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Name of Company :	TABULATED	
HUMET Trade Research and Development Co. **Name of Finished Products :** HUMET Turbo Capsule and Humetta Sparking Tablet **Name of Active Ingredient :** Potassium humate	**STUDY REPORT** **ref. to III.B.110** **Page / Number** **1 / 1**	

REPEATED DOSE TOXICITY	180-Day Repeated Dose Toxicity Study of Dotated Potassium Humate Powder Test Item in Beagle Dogs

Ref. to document : 00/518-107K **Volume:**1-2 **Page :**1 to 40 + Appendices **Addendum No. :** ---
Report date : October 10. 2001 **Number :** **Study period (years) :** 2000

Species/Strain : Beagle dogs / Wobe kennel

Number of animals : 40 **Duration of treatment :** 180 days

Observation period after the end of dosing : 30 days
(Untreated Control and 150mg/kg Dotated Potassium Humate-Powder)

Administration route: oral route

Treatment of controls : non
Group 1: Untreated. on normal food

Age : 6-7 months **at study**
Body weight : 7.9-9.4 kg **initiation**

Treatment days per week : 7

Study group	Untreated Control		Dotated Potassium Humate-Powder 15 mg/kg		Dotated Potassium Humate-Powder 50 mg/kg		Dotated Potassium Humate-Powder 150mg/kg	
Sex (m/f)	f	m	f	m	f	m	f	m
Number of test animals	4+2	4+2	4	4	4	4	4+2	4+2
Number of animals died Or sacrificed in extremis	0	0	0	0	0	0	0	0

Clinical observations :	[x] yes	[ ] no	**Clinical chemistry :**	[x] yes	[ ] no
Food consumption :	[x] yes	[x] no	**Urinalysis :**	[x] yes	[ ] no
Water consumption :	[ ] yes	[x] no	**Organ weights :**	[x] yes	[ ] no
Body weight :	[x] yes	[ ] no	**Necropsy :**	[x] yes	[ ] no
Hematology:	[x] yes	[ ] no	**Histology :**	[x] yes	[ ] no

Additional information: Ophthalmology, ECG and evaluation of bone marrow smears were performed additionally

Conclusions: - Test item administered orally to dogs in doses of 15, 50 and 150mg/kg respectively over 180 days – caused vomiting and diarrhea in 50 and 150 mg/kg dose groups.

-Reversible centrilobular fatty infiltration occurred in the liver (3m+2f) and -diffuse diffuse fatty infiltration was found in the kidney (3m+3f) in the 150 mg/kg dose group. -Myocardial necrosis was observed in the same group in two females. -The 15 mg/kg dose was estimated as NOAEL.

Histology performed according to OECD Note for Guidance : [x] Yes [ ] No

Study conducted by the applicant : [ ] Yes [x] No

If "no", indicate the name and address of the institute that conducted the study
Toxicological Research Centre Ltd. Szabadságpuszta Veszprém POB348 H-8201 Hungary EUROPE

Study in compliance with GLP : [x] Yes [ ] No [ ] Not required

Name of Company : HUMET Trade Research and Development Co. **Name of Finished Product :** HUMET Turbo Capsule and Humetta Sparking Tablet **Name of Active Ingredient :** Potassium Humate	**TABULATED** **STUDY REPORT** **ref.to III.D.210** **Page / Number** **1/2**	
MUTAGENIC POTENTIAL In vivo	MUTAGENIC EFFECT OF DOTATED POTASSIUM HUMATE POWDER TEST ITEM BY MICRONUCLEUS TEST	

Ref. to document :00/518-013M **Volume** **Page :**1 to 15+Appendices **Addendum No. :**
Report date : January 26. 2001 **Number :** **Study period (years) :** 2000

Species/Strain : NMRI mice
Number of animals : 30 males 30 females
Target cells : polychromatic erythrocytes
Test for induction of : micronucleated polychromatic erythrocytes

Treatment of negative control : untreated 5 male and 5 female animals
Vehicle control : Aqua destillate pro inj, (Supplier: Gróf Esterházy Hospital, Pápa Hungary)
Dosage (0.1 ml / 10 g b.w.)
Administration route : by oral route
Treatment schedule : single treatment of 10 male and 10 female animals
Sampling times : 24 and 48 hours after the treatment (5 male and 5 female at each occasion)

Test substance Dotated Potassium Humate Powder, active component: Potassium Humate 22.5 mg/kg
Dosage (2000 mg/kg)
Administration route : by oral route
Treatment schedule : single treatment of 10 male and 10 female animals
Sampling times : 24 and 48 hours after the treatment (5 male and 5 female at each occasion)

Positive control : Cyclophosphamide (Supplier: SIGMA)
Dosage (60 mg/kg) :
Administration route : by intraperitoneal route
Treatment schedule : single treatment of 5 male and 5 female animals
Sampling times : 48 hours after the treatment

Number and sex of animals analysed per group :

Sampling time	Groups			
	Untreated Control	Vehicle Control	Test Item	Positive Control
24 hours	5 M -5 F	5 M -5 F	5 M -5 F	
48 hours		5 M -5 F	5 M -5 F	5 M -5 F

Number of cells analysed per animal : 2000 polychromatic erythrocytes were analysed / animals

Study in compliance with GLP : [x] **Yes** [ ]☐ **No** [ ] **Not required**
Page :Addendum 2

Name of Company : HUMET Trade Research and Development Co. **Name of Finished Product :** HUMET Turbo Capsule and Humetta Sparking Tablet **Name of Active Ingredient :** Potassium Humate	**TABULATED STUDY REPORT** **ref.to III.D.210** **Page / Number** **2/2**	
MUTAGENIC POTENTIAL In vivo	MUTAGENIC EFFECT OF DOTATED POTASSIUM HUMATE POWDER TEST ITEM BY MICRONUCLEUS TEST	
Ref. to document :00/518-013M **Volume** **Report date** : 2001	**Page** :1 to15+Appendices **Addendum No. : Number :** **Study period (years)** : 2000	

Toxic/cytotoxic effects : Considerable differences in the ratio of polychromatic and normochromatic erythrocytes were not found

Genotoxic effects : Dotated Potassium Humate powder did not induce significant increase in the number of micronucleated polychromatic erythrocytes at 2000 mg/kg dose level after single administration in NMRI mice.

Effects of the positive control : Cyclophosphamide treated mice (60 mg/kg) showed significantly increased numbers of micronucleated polychromatic erythrocytes compared to the control.

Additional data regarding methods and time schedule of the test :

Study conducted by the applicant : [ ] **Yes** [x] **No**
If "no", indicate the name and address of the institute that conducted the study :
Toxicological Research Centre Ltd. Szabadságpuszta Veszprém POB348 H-8201 Hungary EUROPE

Study in compliance with GLP : [x] **Yes** [ ]☐ **No** [ ] **Not required**

Page : Addendum 3

Addendum to the "Expert Report on the Pharmaco-Toxicological (Pre-Clinical) Documentation of Humet-R Syrup (1999)

2002.

Compiled by László Német DVM, PhD.

Introduction

The Pharmaco-Toxicological (Pre-Clinical) Documentation of Humet Products (Humet -R Syrup, HUMET Turbo Capsule and Humetta Sparking Tablet) has been amended with two further preclinical GLP studies concerning the genotoxicity and the long term non rodent repeated dose toxicity of the active ingredient potassium humate.

MUTAGENIC EFFECT OF DOTATED POTASSIUM HUMATE POWDER TEST ITEM BY MICRONUCLEUS TEST

(See: Tabulated study report Page Addendum 2-3)

Breakage of chromatids or chromosomes can result in micronucleus formation if an acentric fragment is produced; therefore assays detecting either chromosomal aberrations or micronuclei are acceptable for detecting clastogens.

Unless there are obvious differences in toxicity or metabolism between male and female rodents, then males alone are sufficient for use in bone marrow micronucleus tests.

In the present GLP conform study both sexes were used for the detection of micronucleated polychromatic erythrocytes in bone marrow cells of NMRI mice.

Dotated Potassium Humate powder did not induce significant increase in the number of micronucleated polychromatic erythrocytes after single administration of 2000 mg/kg dose in NMRI mice.

Cyclophosphamide treated mice (60 mg/kg) showed significantly increased numbers of micronucleated polychromatic erythrocytes compared to the vehicle control.

The test item exhibited no genotoxic potency examined in two in vitro tests (in vitro mutgenicity tests on human peripherial lymphocytes and Reverse mutation assay in Salmonella typhimurium strains) and in the in vivo test for chromosomal damage using mice bone marrow cells.

The performed battery of genotoxicity tests fulfils the recommendations of ICH guidelines: STANDARD BATTERY FOR GENOTOXICITY TESTING OF PHARMACEUTICALS and GUIDANCE ON SPECIFIC ASPECTS OF REGULATORY GENOTOXICITY TESTS FOR PHARMACEUTICALS and proves the safety of the test item.

180-DAY REPEATED DOSE TOXICITY STUDY OF DOTATED POTASSIUM HUMATE POWDER TEST ITEM IN BEAGLE DOGS

(See: Tabulated study report Page Addendum 4)

The GLP conform study was performed according to the OECD guidelines 'No.409, Repeated Dose 90-day Oral Toxicity Study in Non-Rodents'.

Test item was administered orally to Beagle dogs of both sexes in doses of 15, 50 and 150 mg/kg respectively over 180 days. Duration of the recovery period was 30 days after the termination of the treatment for control and high dose treated animals.

Dogs were observed daily, body weight was measured weekly, blood samples were taken monthly for clinical chemistry and hematology.

Basic , interim and terminal urinalysis ophthalmology and ECG were employed. After the necropsy organ weights were measured and full histo-pathology was performed. Additionally detailed evaluation of bone marrow smears and histometry of lymphoid organs were carried out.

Vomiting and thin faces was observed during the treatment in a dose dependent manner.

Centrilobular fatty infiltration in the liver and diffuse fatty infiltration occurred in the kidney in the 150 mg/kg dose group in both sexes. Myocardial necrosis was observed in the same group in two female dogs.

No other treatment related changes were found in the examined parameters of the study.

The estimation of NOAEL dose (no observed adverse effect level) after the 180-day treatment was based on the clinical symptoms: vomiting and thin faces and was established in 15 mg/kg/day dose of the test item.

In the 28-day repeated dose study of HUMET-R Syrup in rats NOEL and NOAL were estimated over the oral 50 mg/kg dose, based on the lowered body weights in female rats.

TRC

Toxicological Research Centre Ltd.

Address 8200 Veszprém, Szabadságpuszta
Letters. 8201 Veszprém P O B 348
Phone: /36-88/ 545-300
Fax: /36-88/ 545-301

FINAL REPORT

180-DAY REPEATED DOSE ORAL TOXICITY STUDY OF DOTATED POTASSIUM HUMATE POWDER TEST ITEM IN BEAGLE DOGS

VOLUME 1

2001

STATEMENT OF THE STUDY DIRECTOR

This study has been performed in accordance with the study plan agreed upon by Sponsor, the OECD Guidelines for Testing of Chemicals, "No. 409, Repeated Dose 90-day Oral Toxicity Study in Non-Rodents" (21st Sept., 1998) and The Principle of Good Laboratory Practice (Paris, 1997).

I, the undersigned declare that this report constitutes a true record of the actions undertaken and the results obtained in the course of this study.

Signature:_____ Date:_____

Dr. Zsuzsa Holló
Study Director

STATEMENT OF THE MANAGEMENT

According to the conditions of the research and development assignment between HUMET Ltd. and TOXICOLOGICAL RESEARCH CENTRE Ltd. (as Testing Facility) "180-day repeated dose oral toxicity study of Dotated Potassium Humate Powder test item in Beagle dogs" has been performed, insisting on the GLP requirements.

Signature:_____ Date:_____

Dr. Gábor Hirka
Managing Director

QUALITY ASSURANCE STATEMENT

Study Code: 00/518-107K

Study Title: 180-day repeated dose oral toxicity study of Dotated Potassium Humate Powder test item in Beagle dogs

Test Item: Dotated Potassium Humate Powder

This study has been inspected, and this report audited by the Quality Assurance Unit in compliance with the Principles of Good Laboratory Practice. As far as it can be reasonably established the methods described and the results incorporated in this report accurately reflect the raw data produced during this study.

All inspections, data reviews and the report audit were reported in written form to the study director and to management.

Date	Inspection/audit	Date of report to Management and Study Director
03 Oct. 2000	Study Plan	03 Oct. 2000
11 Oct. 2000	Animal keeping conditions, food Treatment Clinical Observations Measurement of given and remained food	11 Oct. 2000
22 Dec. 2000	Treatment Measurement of given and remained food	22 Dec. 2000
03 Jan. 2001	ECG	03 Jan. 2001
06 Feb. 2001	Animal keeping conditions Body weight measurement Clinical Observations Tools & Equipment	06 Feb. 2001
08 Feb. 2001	Handling of Test Item Capsuling Dosages	08 Feb. 2001
01 Apr. 2001	Ophthalmocopic examinations	01 Apr. 2001
02 Apr. 2001	Blood analysis, Blood sampling Records of Urine analysis Tools & Equipment Necropsy	02 Apr. 2001
24 May 2001	Histopathological processing	24 May 2001
26 July 2001	Draft Report	26 July 2001
10 Oct. 2001	Final Report	10 Oct. 2001

Signature: _____ Date: _____

Ildikó Hermann
Head of QAU

SPONSOR	:	HUMET Ltd. Address: H-1121 Budapest, Konkoly Thege u. 29-33 HUNGARY Phone: 36-1-392-2261 Fax: 36-1-392-2265
STUDY PERFORMED BY	:	TOXICOLOGICAL RESEARCH CENTRE Ltd. Address: H-8201 Veszprém, Szabadságpuszta P.O.B. 348. HUNGARY Phone: (88) 545-300 Fax: (88) 545-301
ON BEHALF OF TRC	:	Dr. Gábor Hirka managing director
STUDY DIRECTOR	:	Zsuzsa Holló, D.V.M. veterinarian
QUALITY ASSURANCE	:	Ildikó Hermann head of QAU
RESPONSIBLE PERSONS		
STUDY MONITOR OF THE SPONSOR	:	Dr. Alice Druga
DEPUTY STUDY DIRECTOR	:	Róbert Lippert animal husbandry engineer
VETERINARY CONTROL AND NECROPSY	:	Levente Zoltán, D.V.M. veterinarian
HAEMATOLOGY AND CLINICAL CHEMISTRY	:	Enikő Pápai, .D. M. haematologist
HISTOPATHOLOGY	:	Róbert Glávits, D.V.M., PhD. histopathologist
STATISTICAL DATA PROCESSING	:	Márta Tenk deputy head of data processing unit

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STUDY SCHEDULE

PRETREATMENT PERIOD

Animal received	: 11, September 2000
Veterinary control	: 12, September 2000
Body weight measurement	: 12, 29 September and 03 October 2000
Randomization	: 29 September, 2000
Urine sampling	: 26-29 September, 2000
ECG recording	: 26-28 September, 2000
Ophthalmoscopic examination	: 26-28 September, 2000
Blood sampling for haematology and clinical chemistry	: 27 28 September, 2000

TREATMENT PERIOD

Days of treatment	: from 04 October., then daily 180 days consecutively, 2000
Body weight measurement	: from 03 October , then weekly, 2000
Food consumption	: from 05 October, then daily, 2000
Clinical observation	: from 04 October, then twice daily, 2000
Mortality	: from 04 October, then twice daily, 2000
Urine sampling	: 03-05 January, 27-29 March, 2001
ECG recording	: 03-04 January, and from 31 March, to 01 April, 2001
Ophthalmoscopic examination	: from 31 March, to 01 April, 2001
Blood sampling for haematology and clinical chemistry	: 03 November and 03 December, 2000 02 January, 01 February, 03 March, 02 April, 2001
Necropsy	: 02 April, 2001

RECOVERY PERIOD

Days of recovery period	: from 02 April to 02 May, 2001
Body weight measurement	: from 02 April, then weekly, 2001
Food consumption	: from 02 April, then daily, 2001
Clinical observation	: from 02 April, then twice daily, 2001
Mortality	: from 02 April, then twice daily, 2001
Urine sampling	: 01 May, 2001
ECG recording	: 30 April, 2001
Ophthalmoscopic examination	: 30 April, 2001
Blood sampling for haematology and clinical chemistry	: 02 May, 2001
Necropsy	: 02 May, 2001

TABULATED SUMMARY

DOSAGE mg/kg	Placebo	15 mg/kg/day	50 mg/kg/day	150 mg/kg/day
Male and Female	2 x (4+2)	2 x 4	2 x 4	2 x (4+2)
Clinical signs	-	NTD	Rarely vomiting and thin faeces	Vomiting., thin faeces and diarrhea
Body weight gain	-	NTD	NTD	NTD
Food consumption	-	NTD	NTD	NTD
Haematology	-	NTD	NTD	NTD
Clinical chemistry	-	NTD	NTD	NTD
Urinalysis	-	NTD	NTD	NTD
EKG data	-	NTD	NTD	NTD
Ophthalmology	-	NTD	NTD	NTD
Pathology	-	NTD	NTD	NTD
Organ weight	-	NTD	NTD	NTD
Histopathology	-	NTD	NTD	-centrolobular, slight, fine fatty infiltration in the liver -diffuse slight, fine droplet fatty infiltration in the kidneys -zonal decrease in glycogen content of hepatocytes -acute focal myocardial necrosis -subacute focal myocardial necrosis
Conclusions	In our experimental conditions the test item Dotated Potassium Humate Powder caused: - vomiting and thin faeces (diarrhea) in 50 and 150 mg/kg dose groups - slight degree reversible fine droplet centrolobular fatty infiltration of the liver in three male and two female animals in the 150 mg/kg dose groups, and fine droplet diffuse fatty infiltration in the kidney in three female and three male animals in the 150 mg/kg dose groups. In additon, not provable, but can not be excluded that the long term rationing of the high dose of test item played role in the pathogenesis of acute focal myocardial necrosis in one female animal and of subacute focal myocardial necrosis in another female animal. **Under the present experimental conditions the NOAEL -no observed adverse effect level- is 15 mg/kg/day.**			

Remark: NTD = no treatment-related differences

SUMMARY

The aim of the study was the evaluation of the toxic characteristics of the test item Dotated Potassium Humate Powder administered in repeated daily oral doses for 180 days.
The doses were chosen on the basis of the results of the preliminary study. The established doses were 15, 50 and 150 mg/kg/day for 180 days.
Dose groups and control group were involved in the study in both sexes. Four animals of both sexes/groups were used, except the control and high dose group where the groups were completed with 2 recovery animals in both sexes.
The test item was applied daily (on a 7-days/week basis) by oral application, in gelatine capsule.
The checks of mortality, and the clinical observation were performed twice daily, the non-consumed food weighing was done daily.
The body weight of the animals was measured once a week.
The haematological and clinical chemical investigations were performed prior to the treatment than monthly, at the end of the study and at the end of the recovery period.
Additional blood samples were taken from all animals from the control and high dose groups at the end of the first and third month and at the end of the treatment period for trace element level determination (5 ml blood were collected from each animal in Vacutainer Brand Evacuated Blood Collection tubes Hemogard NH). The trace element level determination will be reported separately by the Sponsor.
The ophthalmoscopic examinations were performed prior to the treatment, at the end of the treatment period and at the end of the recovery period.
The ECG recording and urinalysis were performed prior to the treatment at midway, at the end of the treatment period and at the end of the recovery period.
Terminally gross necropsy and sampling for histopathological examination were performed according to the study plan. The sections were stained with haematoxilin-eosin and the frozen sections of the liver and kidneys were stained with Oil-Red-O method. The liver, kidneys and heart sections were stained by PAS method, too.
The following results were obtained:

Mortality

No animals died during the study.

Clinical signs

In the **control** groups once diarrhea was observed.

In the **15 mg/kg** dose of the test item Dotated Potassium Humate Powder in 3 cases vomiting and in 2 cases thin faeces occurred.

In the **50 mg/kg** dose of the test item Dotated Potassium Humate Powder in 9 cases vomiting and in 8 cases thin faeces were recorded.

In the **150 mg/kg** dose of the test item Dotated Potassium Humate Powder 127 cases vomiting and in 51 cases diarrhea or thin faeces were noticed.

Body weight

No biologically significantly deviation was found.

Food consumption

Statistically significantly deviation was not found.

ECG investigation

No direct treatment related ECG alterations were found.

Ophthalmoscopic examination

No treatment related ophthalmological alterations were found during the study.

Haematology, clinical chemistry and urinalysis

The applied dose levels of Dotated Potassium Humate Powder did not cause any severe changes of the hematological and clinical chemical parameters during the 180-day oral administration, which could refer to the injury of any organs of vital importance.
The test item had a slight suspected, but not proved, protein, albumin and cholesterol decreasing effect in both sexes mostly in the high dose groups and a slight calcium decreasing effect in the females of the high dose group. The decreased values remained between the physiological ranges.

Necropsy

Macroscopic alterations in direct connection with the toxic effect of the test item Dotated Potassium Humate Powder were not found.

Organ weight

Organ weight value difference in direct connection with the toxic effect of the test item Dotated Potassium Humate Powder was not detected.

Histopathology

The test item Dotated Potassium Humate Powder caused slight degree reversible fine droplet centrolobular fatty infiltration of the liver in three male and two female animals in the 150 mg/kg dose groups, and fine droplet diffuse fatty infiltration in the kidney in three female and three male animals in the 150 mg/kg dose groups.
The zonal decrease in glycogen content of hepatocytes in some animals belonging to the 150 mg/kg dose groups seems to be the consequence of the metabolic charging effect of the high dose of test item.
In additon, not provable, but can not be excluded that the long term rationing of the high dose of test item played role in the pathogenesis of acute focal myocardial necrosis in one female animal and of subacute focal myocardial necrosis in one other female animal.

Bone marrow smears

Under our experimental conditions the test item Dotated Potassium Humate Powder did not influence the bone marrow function.

Histometry

With the histometrical examination of the lymphoid organs neither hyperplastic nor regressive alterations were detected in any dose groups, referring to an immunostimulating or immunosuppressive effect of the test item.

CONCLUSION

The test item marked Dotated Potassium Humate Powder administered orally to dogs in doses of 15, 50 and 150 mg/kg respectively over 180 days clinically caused vomiting and thin faeces (diarrhea) in 50 and 150 mg/kg dose groups.
Histopathologically in our experimental conditions the test item Dotated Potassium Humate Powder caused slight degree reversible fine droplet centrolobular fatty infiltration in the liver of three male and two female animals in the 150 mg/kg dose groups, and fine droplet diffuse fatty infiltration in the kidney of three female and three male animals in the 150 mg/kg dose groups. In addition, not provable, but can not be excluded that the long term rationing of the high dose of test item played role in the pathogenesis of acute focal myocardial necrosis in one female animal and of subacute focal myocardial necrosis in one other female animal.

Under the present experimental conditions the NOAEL -no observed adverse effect level- is 15 mg/kg/day.

1. INTRODUCTION

The objective of the study was the evaluation of the toxic characteristics of the test item Dotated Potassium Humate Powder administered in repeated daily oral administration at various dose levels for 180 days.
The study provided information on the target organ toxicity, on the "no observable adverse effect level" (NOAEL).

2. MATERIALS AND METHODS

2.1. TEST ITEM

Name:	Dotated Potassium Humate Powder
Appearance:	brown powder
Storage:	ambient 22±2 °C
Batch No.:	007DKH0600
Date of production:	19 June 2000
Expiration:	one year

2.1.2. Vehicle

Capsule used in the study:	gelatin capsule, size 13 TORPAC USA 52. Seftron Circle, Pistcataway N.Y. 08854

2.1.1. Identification and receipt

The test item of a suitable chemical purity was supplied by the Sponsor. All precautions required in the handling and disposal of the test item was outlined by the Sponsor. Analytical certificate was supplied by the Sponsor and will be archived with the raw data. In our condition we had no possibility to made control analysis for the chemical identification of the test item.

2.2. EXPERIMENTAL ANIMALS

Species and strain:	Beagle dogs
Source:	"WOBE KENNEL" H - 1163. Budapest, Színjátszó u. 15. HUNGARY
Justification of the strain:	The Beagle dog as a nonrodent, is a standard and preferred experimental animal. It has a large amount of historical background data.

Number of animals employed in the study:	20 animal/sex
Number of reserve animals for selection, randomization:	4 animal/sex (these animals were excluded and eliminated before study)
Age of animals:	between 6-7 months
Quarantine and acclimatization period:	23 days

2.2.1. Vaccinations

Certificates from type and time schedule of vaccinations were given by the breeder and will be archived with the raw data.

2.2.2. Hygiene

Animals used were under a continuous veterinary control, they were vaccinated and cleansed of vermin, as certified by the breeder. The dogs did not receive vermicide during the study.
The last vermicide treatment was performed at TRC Ltd. 22 days prior to the first treatment.
Vermicide: Drontal Plus (Bayer AG, Leverkusen).

2.2.3. Husbandry

Animal health:	Only animals in acceptable health condition were used for the test. The veterinarian certified it.
Room:	1
Housing:	individual caging
Size of cage:	1.2 square meter
Light:	12 hours daily, from 6 a.m. to 6 p.m.
Temperature:	20 ± 8 °C
Relative humidity:	30 - 80%

There was an indoor-outdoor system with directed running. The dogs were kept at least 2-3 hours daily in the runway (13 square meter/4-6 dogs bitumen-covered surface). The individual cages were cleaned daily, the runways were cleaned at least in every second day.

2.2.4. Food and feeding

The animals received SSNIFF diet for dogs produced by SSNIFF GmbH D-59494 Soest, Germany. The food was offered daily 300 g/dog at noon (from noon to 8 a.m. next day). The food consumption was determined daily by weighing the non-consumed diet (with precision of 1 g).
The diet was analysed for possible contamination (aflatoxine, pesticide residues, and heavy metal) once during the study. Ingredients and microbiological sampling were also done. The obtained data will be archived with the raw data at TRC.

2.2.5. Water supply

The animals received tap water, as for human consumption, ad libitum.
The quality control of water was performed by Central Laboratory "Bakonykarszt" (H-8200 Veszprém, Pápai str. 41. Hungary). The quality control results are available at TRC.

2.2.6. Identification

The individual identification was performed by tattoo numbers on ears.

2.2.7. Randomization

Animals were allocated to treated and control groups randomly by body weight. The grouping was checked by SPSS/PC+ computer program according to the actual body weight verifying the homogeneity and deviations among the groups.

2.3. ADMINISTRATION OF THE TEST ITEM

2.3.1. Dosages

Justification of the doses:
Doses were chosen on basis of preliminary study.
Dose levels and applications:
Three different dose groups were used.
One dose level was included which not cause any evidence of toxicity (no-toxic-effect level) and one dose level which produce overt toxic effects. In order that the complete picture of toxicity of the test item to be revealed, it was desirable to set the dose level so that a dose-response relationship could be achieved. In addition, vehicle control groups were included.

The following groups and dose levels were used.

Groups	Number of animals		Dose
	male	female	mg/kg
0 (control)	4+2	4+2	placebo
1	4	4	15
2	4	4	50
3	4+2	4+2	150

The test item was administered once daily before feeding at 7-9 a.m. on seven days per week basis. The dosages were calculated weekly according to body weight gain of the animals. The doses were adjusted according to body weight on the day following the body weight measurement. The test item was prepared individually for the animal into gelatine capsules. Treatment was carried out orally placing the gelatine capsule on the root of the tongue.
The control animals were treated in the same manner with placebo gelatine capsules.

Duration:

The test item was administered to the animals for 180 days. Duration of the recovery period was 30 days.

2.4. OBSERVATIONS

2.4.1. Clinical Observations

All animals were observed daily with regard to their sensory function and behaviour, hair coat, body orifices, urine and faecal excretion, for their general health status and dose responses. The observations were carried out twice daily and were recorded on individual data sheets.

Observations of mortality: twice daily.

2.4.2. Body Weight

Body weight was recorded individually for each animal on the day of receiving, before randomisation, one day before starting the treatment, weekly and at the end (on the day of necropsy) with a precision of 100 g.

2.4.3. Food Consumption

The non-consumed diet was weighed every day from the second treatment day. From this data, the average food consumption was calculated weekly.

2.4.4. ECG Examinations

ECG was recorded with EC60 SILOGIC apparatus from each animal at the beginning, at midway and at termination of the study and at the end of the recovery period.
The ECG was recorded in 6 leads (I, II, III, aVr, aVl, aVf), the measurements were performed in the lead II, which were evaluated statistically.

2.4.5. Ophthalmoscopic Examinations

Examinations were performed by KOWA RC-2 ophthalmoscope.
10 minutes prior to the ophthalmoscopy one drop of 0.5 % Mydrum (N-aethyl/gamma-picolyl/-tropamid) was applied in the corner of both eyes.
Cornea, conjunctiva, episcleral vessels, anterior chamber, pupil, lens, retina, optic disc were examined in all animals at the beginning, at termination of the study and at the end of the recovery period.

2.5. LABORATORY EXAMINATIONS

Haematology and Clinical Chemistry

Blood collection

Blood samples were collected from each animal for analysis before first treatment day, monthly, terminally and at the end of the recovery period.

Blood sampling: from antibrachial cephalic vein.
Anticoagulants to be used: EDTA (for haematology)
Sodium citrate (for PTT, PT)

Investigational Parameters

a., Haematology

PARAMETERS / UNITS OF MEASURE	METHODS	EQUIPMENT
RBC Red Blood Cell (erythrocyte) count, 10^{12}/l	Aut. cell count	Serono Baker System 9120+
WBC White Blood Cell (leukocyte) count, 10^{9}/l	Aut. cell count	Serono Baker System 9120+
Hgb Hemoglobin concentration, mmol/l	Determined from the absorbance	Serono Baker System 9120+

PARAMETERS / UNITS OF MEASURE	METHODS	EQUIPMENT
Htc Hematocrit (relative volume of erythrocytes)l/l	Computed by equipment	Serono Baker System 9120+
MCV Mean Corpuscular (erythrocyte) Volume, fl	Derived from the RBC histogram	Serono Baker System 9120+
MCH Mean Corpuscular (erythrocyte) Hemoglobin, f mmol/l	Computed by equipment	Serono Baker System 9120+
MCHC Mean Corpuscular (erythrocyte) Hemoglobin Concentration, mmol/l	Computed by equipment	Serono Baker System 9120+
RDW Red blood cell (erythrocyte) Distribution Width, %	Derived from the RBC histogram	Serono Baker System 9120+
Plt Platelet (thrombocyte) count, 10^9/l	Derived from the Plt fitted curve	Serono Baker System 9120+
MPV Mean Platelet (thrombocyte) Volume, fl	Derived from the Plt histogram	Serono Baker System 9120+
PTT Partial Thrombo-plastin Time, sec	Kaoline method	ST-ART4 Coagulometer
PT Prothrombin Time, sec	Quick method (Biggs, R., and R.G. MacFarlane (1962.) Human Blood Coag. and its Disorders, Oxford)	ST-ART4 Coagulometer
Differential white blood cell count, %	Counting blood smears stained according to Pappenheim	Leitz Microscope
Reticulocytes, %	With brillantcresyl blue staining	Leitz Microscope

b., Clinical Chemistry

PARAMETERS / UNITS OF MEASURE	METHODS	EQUIPMENT
Glucose Blood sugar conc., mmol/l	Colorimetric test (540 nm)	VITROS 250
T. Bil **Total** Billirubin conc., μmol/l	End-point colorimetric (dual-welength) test (400 & 460 nm)	VITROS 250

PARAMETERS / UNITS OF MEASURE	METHODS	EQUIPMENT
Urea Nitrogen Urea conc., mmol/l	Colorimetric test (670 nm)	VITROS 250
Chol. Cholesterol conc., mmol/l	Colorimetric test (540 nm)	VITROS 250
Creat. Creatinine conc., µmol/l	Two-point rate test (670 nm)	VITROS 250
Phos. Phosphorus conc., mmol/l	Colorimetric test (680 nm)	VITROS 250
Na^+ Sodium conc., mmol/l	Potentiometric test	VITROS 250
K^+ Potassium conc., mmol/l	Potentiometric test	VITROS 250
Ca^{++} Calcium conc., mmol/l	Colorimetric test (680 nm)	VITROS 250
Cl^- Chloride conc., mmol/l	Potentiometric test	VITROS 250
Tot. prot. Total Protein conc., g/l	Colorimetric test (540 nm)	VITROS 250
Alb. Albumin conc., g/l	Colorimetric test (630 nm)	VITROS 250
A/G Alb/glob ration	Calculated value	
AST/GOT, Aspartate Aminotransferase activity, U/l	Multiple-pointrate test (340 nm)	VITROS 250
ALT/GPT Alanine Aminotransferase activity, U/l	Multiple-point rate test (340 nm)	VITROS 250
GGT Gamma Glutamyltransferase – activity, U/l	Multiple-point rate test (400 nm)	VITROS 250
ALP Alkaline. Phosphatase – activity, U/l	Multiple-point rate test (400 nm)	VITROS 250
LDH Lactate-dehydrogenase activity, U/l	Multiple-point rate test (340 nm)	VITROS 250

Additional blood samples were taken from all animals from the control and high dose groups at the end of the first and third month and at the end of the treatment period for trace element level determination.
5 ml blood was collected from each animal in Vacutainer Brand Evacuated Blood Collection tubes Hemogard NH (Becton Dickinson).
The blood samples were stored at -22 ±2 C in TRC before they were transferred to the Sponsor. The trace elements analysis results will be separately reported by the Sponsor.

c., Urinalysis

Urine sampling and investigations were made prior to the treatment, at midway, at termination and at the end of the recovery period.
Urine samples were collected in metabolism cages.

PARAMETERS	METHODS	EQUIPMENT
Leucocytes	Combur^{10} test M	MIDITRON JUNIOR
Nitrit	Combur^{10} test M	MIDITRON JUNIOR
pH	Combur^{10} test M	MIDITRON JUNIOR
Protein	Combur^{10} test M	MIDITRON JUNIOR
Glucose	Combur^{10} test M	MIDITRON JUNIOR
UBG	Combur^{10} test M	MIDITRON JUNIOR
Bilirubin	Combur^{10} test M	MIDITRON JUNIOR
Keton	Combur^{10} test M	MIDITRON JUNIOR
Blood	Combur^{10} test M	MIDITRON JUNIOR
Spec. gravity	Gravimetric method	Sartorius H51D Balance
Sediment	Microscopic examination	Leitz Microscope
Volume	Volumetric method	Measuring cylinder

2.6. PATHOLOGY

2.6.1. Necropsy

Four animals from each dose group were sacrificed at the end of the treatment by i.v. injection of Nembutal following the last dose administration. The animals received their last dose one day prior to necropsy.
The blood was removed by cutting the carotid artery and a complete autopsy was performed.

The recovery animals were retained for 4 weeks recovery period and were observed similarly to the animals in the dosing period, then they were sacrificed similarly, too. The following observations were made and recorded in the post-mortem record sheets after the examination of the external appearance: the cranial, thoracic and abdominal cavities were opened and the appearance of the tissues and organs were observed, and any abnormality was recorded with details of the location, colour, shape and size.

All organs were removed and preserved in 10 % buffered formol saline, as follows:

Adrenals	Muscle (m. quadriceps femoris)
Aorta (thoracic and abdominal)	Oesophagus
Bone marrow (os femoris)	Ovaries
Caecum	Pancreas
Cerebellum	Pituitary
Cerebrum	Prostate
Colon	Rectum
Duodenum	Salivary gland
Epididymides	Sciatic nerve
Eyes	Skin
Gall bladder	Spinal cord
Gross lesion	Spleen
Heart	Sternum
Ileum	Stomach
Jejunum	Submandibular lymph node
Kidneys	Testes
Lacrimal glands	Thymus
Liver	Thyroids + parathyroids
Lungs	Trachea
Lymph node (mesenteric)	Urinary bladder
Mammary gland	Uterus
Medulla oblongata	Vagina

2.6.2. Determinations of Organ Weights

The following organ weights were determined and recorded from all animals:

Adrenals (l. + r.)	Liver
Thyroids + Parathyroids (l. + r.)	Testes (l. + r.)
Brain	Kidneys (l. + r.)
Pituitary	Spleen
Ovaries (l. + r.)	Thymus

l = left, r = right

In addition bone marrow smears from femur was prepared and evaluated, too.

2.6.3. Histopathology

Histological examination was performed on the preserved organs of all animals. The sections were stained with hemotoxilin-eosine and evaluated by means of a light microscope. From the liver, kidneys and heart the sections were stained by PAS method, too. The frozen sections of the liver and kidney were stained by Oil-Red-O to detect lipids.

2.6.4. Histometry

In the case of the lymphoid organs (thymus, spleen, lymph nodes and the gut associated lymphoid tissue-GALT) histometric examination was carried out as well. The aim of the examination was to demonstrate the size of the zone of the B- and T-dependent lymphocytes in the different lymphoid organs.

The following objects, were measured:

in the case of the THYMUS:	the transverse diameter of the lobules the transverse size of the cortex of the lobules
in the case of the SPLEEN:	the diameter of the follicles the diameter of the periarteriolar lymphoids sheath (PALS)
in the case of the GALT:	the transverse diameter of the follicles
in the case of the lymph nodes:	the diameter of the secondary follicles the size of the paracortical zone

2.7. STATISTICAL EVALUATION

Statistical analysis was performed by SPSSPC+ (4.0.1) software package for the following data:

- body weight data
- food consumption data
- organ weight data
- ECG data
- haematological, clinical chemistry, urine data.

The heterogenity of variance between groups was checked by Bartlett's test.

Where no significant heterogenity was detected, a one-way analysis of variance was carried out. If the obtained result was positive, Duncan's Multiple Range test was used to assess the significance of intergroup differences. In evaluation of the study results the significances between the control and treated groups were considered. In the summarised data the significant differences only between the control and treated groups are indicated.

Where significant heterogenity was found, the normal distribution of data was examined by Kolmogorow-Smirnow test. In case of not normal distribution, the non-parametric method of Kruskal-Wallis One-Way analysis of variance was applied. If positive result was detected, the intergroup comparison was performed using Mann-Whitney U-test.

3. ARCHIVES

The study documents:

- study plan and any amendments,
- all raw data,
- specimen of the test item,
- study report and any amendments,
- biological samples
- correspondence

will be stored in the archives of TRC Ltd. Hungary 8201 Veszprém, Szabadságpuszta P.O.B. 348. according to the OECD GLP and to the TRC's SOPs.

4. REFERENCES

Guidelines for toxicity tests for food and color additives used in food, Chapter IV, FDA, 1982
Draft Redbook II, Guidelines for toxicity tests- Subchronic toxicity tests with Rodents and Non -Rodents- FDA, 1993

5. DEVIATION FROM THE STUDY PLAN

The jejunum and the epididymides were preserved and were examined histopathologically and the lungs' organ weight was measured at necropsy, too.
From the animal (No.907) the bone marrow slide was not evaluated due to technical failure.

6. RESULTS

6.1. CLINICAL OBSERVATIONS

No animals died during the study.

Males

In the **control** group in one case diarrhea was observed.

In the **15 mg/kg** dose of the test item Dotated Potassium Humate Powder in the animal No. 212 in one case faeces content vomiting, in other two cases thin faeces occurred and on 70 consecutive days peanut and pea sized formation was observed on the left costal arch region. This alteration histopathologically was not evaluated.

In the **50 mg/kg** dose of the test item Dotated Potassium Humate Powder in three cases (No. 215. No. 826 and No. 207) vomiting and in two cases (No. 207 and No. 215) thin faeces were observed. These clinical signs were observed from 20 minutes to 2 hours after treatment.

In the **150 mg/kg dose** of the test item Dotated Potassium Humate Powder the clinical signs appeared from 30 minutes to 6 hours after treatment. Mostly foamy, test item content vomiting was noticed altogether in 30 cases, involving all animals. In 24 cases thin faeces or diarrhea was noticed in four animals (No. 171, No. 228, No. 222 and No. 225). In the animal No. 222 on seven consecutive days alopecia was noticed on the left iliacal region, thereafter on this animal on the left abdominal region on 30 consecutive days 3-cm diameter alopecia was observed, too.

See Appendix 1.1 page (1-2) and 2.1 page (1-2)

Females

In the **control** group clinical sign was not observed.

In the **15 mg/kg** dose of the test item Dotated Potassium Humate Powder in the animal No. 926 in two cases vomiting was noticed.

In the **50 mg/kg** dose of the test item Dotated Potassium Humate Powder the clinical signs were observed from 40 minutes to 3 hours after treatment.
In each animal rarely or once, altogether in six cases foamy, or foamy and test item content vomiting occurred. In six cases (in case of the animals No. 725, No. 924 and No. 834) thin faeces was observed.

In the **150 mg/kg dose** of the test item Dotated Potassium Humate Powder the onset of the clinical signs was from 20 minutes to 3 hours 30 minutes after treatment. In all animals, altogether in 94 cases vomiting and in 30 cases thin faeces or diarrhea was observed. The vomiting occurred mostly after administration of the test item, had foamy character, and often contained the test item. Not often faeces content vomiting was observed, too. The presence of the test item in the thin faeces was rarely recognisable. In one case salivation (No. 860), in another case kyphotic posture (No. 880) was noticed. In the animal No. 880 on 15 consecutive days on the left posterior mammary gland nut-sized, dark with irregular surface formation was observed.

See Appendix 1.1 (page 1-2) and 2.1 (page 3-4)

6.2. BODY WEIGHT

Males

In the 24th week the body weight gain data were statistically significantly increased in the low dose group compared to the control group.

See Appendix 1.2 (page 1-5) and 2.2 (page 1-5)

Females

In high dose group in the 8th week the body weight gain data were statistically significantly lower than in the control group. In the 15th and 24th weeks the body weight gain data in the low dose group were statistically significantly higher than in the control groups and in the 25th week and in the low and middle dose groups a slight decrease in the body weight gain data was observed. Altogether at the end of the study statistically significant deviation in the body weight gain was not observed.

See Appendix 1.2 (page 6-10) and 2.2 (page 6-10)

6.3. FOOD CONSUMPTION

The 300 g/dog food was offered daily at noon and the food consumption was determined daily by weighing the non-consumed diet (in the next day morning). Statistically significant deviation in the food consumption was not observed.

See Appendix 1.3 (page 1-6) and 2.3 (page 1-28)

6.4. HAEMATOLOGY

Base level

Males and Females

In males no significant changes in the haematological parameters occurred. In females the MCHC values in the dose group of 50 mg/kg and the MCH values in all dose treated groups were higher than in the control group. These changes were within the physiological range.

End of first month

Males and Females

In males there was a minimal statistically significant elevation in reticulocyte count in the dose group of 150 mg/kg as compared to the control group. In females there was a statistically significant decrease in segmented neutrofil count along an increase in lymphocyte count in 50 mg/kg dose group compared to the control group. The RDW value in dose group of 50 mg/kg and 150 mg/kg showed a statistically significant decrease as compared to the control group. These statistically significant changes were within the physiological range and were not biologically significant.

End of second month

Males and Females

There were no significant changes in males. In females of 15 mg/kg and 50 mg/kg dose groups the segmented neutrophil count showed statistically significant decrease to control group. These changes were not biologically significant.

End of third month

Males and Females

There were no significant changes in males. In females the RDW values in the 150 mg/kg dose group decreased statistically significantly as compared to the control group. This change was within the physiological range and biologically had no significance.

End of fourth month

Males and Females

In males the 15 mg/kg dose group the stab cells and the monocytes were statistically significantly increased compared to the control group. In females the segmented neutrophil count in the low dose group was statistically significantly lower than in the control group, the lymphocyte count was statistically significantly higher in the low dose group, than in the control group and the stab cell count in 50 mg/kg dose group showed significantly higher values than in the control group, too. These changes had no biological significance and the blood smear evaluation did not reveal any pathological findings.

End of fifth month

Males and Females

The MCV value in the 150 mg/kg dose males was statistically significantly decreased as compared to the control group. The PT value in 150 mg/kg dose males was statistically significantly decreased as compared to the control group. In females eosinophil cell count showed statistically significant increase in 50 mg/kg and 150 mg/kg dose groups compared to the control group. These statistically significantly changes were not biologically significant and they were within the physiological range.

End of sixth month

Males and Females

In males statistically significant differences between the treated groups and the control group were not observed. In the females the MCV, MCH, MCHC values showed statistically significant increase in the 50 mg/kg dose groups and the MCH, MCHC values in the 15 mg/kg were increased as compared to the control groups. In the females the RDW values decreased statistically significantly in 150 mg/kg dose group compared to the control. The PTT value showed statistically significant decrease in the 150 mg/kg and 50 mg/kg dose groups compared to the control group. These statistically significant changes were within the physiological range and had no biological significance.

End of recovery period

Males and Females

In the males the MCV values showed statistically significant decrease in the high dose group compared to the control group. This decrease biologically was not significant. In females no significant changes occurred.

See Appendix 1.4 (page 1-32) and 2.4 (page 1-32)

6.5. CLINICAL CHEMISTRY

Base level

Male and Female

In males LDH activity was statistically significantly lower in the proposed 15 mg/kg and 150 mg/kg dose groups as compared to the proposed control group, that has no diagnostical value. In females no significant changes occurred.

End of first month

Males and Females

The bilirubin concentration showed statistically significant increase in 15 mg/kg dose group as compared to the control group without biological meaning.
In females the calcium concentration showed statistically significantly increase in the 15 mg/kg dose group compared to the control group. The sodium and the total protein concentration decreased statistically significantly in the 150 mg/kg dose group as compared to the control groups. These statistically significant changes were within the normal limits.

End of second month

Males and Females

In males there were no significant changes. In the females the calcium concentration showed a statistically significant decrease in 150 mg/kg dose group as compared to the control group. The cholesterol concentration decreased statistically significantly in the high and middle dose females compared to the control females. The total protein concentration showed a statistically significant decrease in female of 150 mg/kg dose group compared to the control group. The albumin concentration also decreased statistically significantly in 150 mg/kg dose and in the low dose groups as compared to the control group. The A/G value was statistically significantly lower in high dose females compared to control females. The LDH activity was statistically significantly increased in 15 mg/kg dose group as compared to the control group. All these statistically significant changes were within the physiological range.

End of third month

Males and Females

In males calcium concentration showed statistically significant decrease in 15 mg/kg and 150 mg/kg dose groups compared to the control group and the carbamide concentration showed a slight decrease in the 50 mg/kg dose group compared to the control group, too. These statistically significant changes were within the physiological range. In females statistically significant differences comparing betweenthe treated groups and control group were not detected.

End of fourth month

Males and Females

The calcium concentration decreased statistically significantly in the 150 mg/kg dose group as compared to the control group. The total protein decreased statistically significantly in the 150 mg/kg dose group as compared to the control group. The A/G value was statistically significantly lower in the 15 mg/kg dose group as compared to the control. The total protein concentration in the high dose group was tending toward the lower limit of the physiological range, the other parameters were within the physiological range. In females there were no significant changes.

End of fifth month

Males and Females

In the males potassium concentration showed a statistically significant decrease in 15 mg/kg dose group compared to control group. The calcium, the sodium and the glucose concentrations showed a statistically significantly decrease in 150 mg/kg dose group as compared to the control group. The LDH activity was statistically significantly higher in 50 mg/kg dose group compared to the control and other treated groups which can be attributed to the increased value of the animal No. 826. The Ldh activity was statistically significantly higher in the 150 mg/kg dose group compared with the control group, too. In females the glucose, sodium, calcium, total protein and albumin concentration showed a statistically significantly decrease in 150 mg/kg dose group as compared to the control group. The ALP activity was statistically significantly higher in the control group compared to all treated dose groups. These statistically significant changes were within the physiological ranges.

End of sixth month

Males and Females

In the males the chloride concentration was statistically significantly higher in the low and high dose groups as compared to the control group. The LDH activity was statistically significantly lower in the low dose group than in the control group, without biological significance. In females the calcium concentration showed a statistically significant decrease in the 150 mg/kg dose group as compared to the control. The ALP activity was higher in the control group compared to the treated groups that can be attributed to the value of the animal No. 864. All these statistically significant changes were within the physiological range.

End of recovery period

Males and Females

In males there were no significant changes. In females the sodium concentration showed a statistically significant increase in the high dose group compared to the control group and the chloride concentration decreased statistically significantly in the treated group as compared to the control group. These statistically significant changes were within the physiological ranges.

See Appendix 1.5 (page 1-32) and 2.5 (page 1-32)

6.6. URINALYSIS

Base level

Males and Females

In males there were no significant changes. In the females of 15 mg/kg and 50 mg/kg dose groups the urine volume was statistically significantly lower compared to the female control group.

Midway

Males and Females

There were no significant changes.

Sixth month

Males and Females

There were no significant changes.

End of recovery period

Significantly changes did not occur neither in males, nor in females.

See Appendix 1.6 (page 1-8) and 2.6 (page 1-8)

6.7. ECG DATA

Males

Statistically significant differences between the values of the treated groups with the control group were not observed.

See Appendix 1.7 (page 1-4) and 2.7 (page 1-4)

Females

At base level the QT interval in the low dose group was statistically significantly shorter than in the control group, while the heart rate showed inverse statistical significant deviation. The QT interval varies inversely with the heart rate, therefore it had no biological meaning. At termination of the study the PQ interval was statistically significantly longer in the middle dose group compared to the control group. These values were within the physiological ranges.

See Appendix 1.7 (page 5-8) and 2.7 (page 5-8)

The incidentally observed alterations in both sexes and in all groups as slight electrical alternans (No. 214 at base level and midway, No. 227 at base level and at termination, No. 050 at base level, No. 159 at base level, No. 225 at midway, No. 891 at base level, No. 828 at base level and at midway, No. 880 at base level, No. 911 at midway) such as ST elevation (No. 214 at base level), sinus arrest (No. 214 at midway, No. 885 at base level, No. 160 at midway and termination, No. 171 at base level, No. 222 at termination, No. 858 at midway and at termination, No. 926 at midway and at termination, No. 725 at termination, No. 889 at termination, No. 860 at base level, No. 911 at termination and at the end of the recovery period), right deviation, negative axis values (No. 035, No. 904), tachycardia (No. 825, No. 875, No. 926 at base level) occurred sporadically.

6.8. OPHTHALMOLOGICAL DATA

Males and Females

In the control female No. 907 at the end of the study in the left lens ball-like filiform formation occurred, this formation was present at the end of the recovery period, too. In case of the animal No. 212 (male) the tapetum nigrum was unpigmented at base level and at termination, too. In this animal the position of the optic disc was situated in the tapetum nigrum. In the control male No. 227 at base level in the vitrous humour haemorrhage was observed.

6.9. PATHOLOGY

In the male **control** group in three cases (No.163, No. 209 and No. 885) pulmonary emphysema occurred. In three animals (No. 209, No. 214 and No. 885) sporadic miliary yellowish-brown foci were detected in the liver.

In the male **recovery control** group in one case (No. 227) pulmonary emphysema occurred.

In the male **15 mg/kg** dose of the test item Dotated Potassium Humate Powder in two cases (No. 160 and No. 226) pulmonary emphysema was found. In one animal (No. 212) 1 cm diameter haematoma was observed in the left side in the lungs. In this animal in the cardiac lobe a 3 cm long inflammation area occurred. On the left side between the 4^{th} and 5^{th} costal arch vital reaction was found.

In the male **50 mg/kg** dose of the test item Dotated Potassium Humate Powder in one case (No. 215) pulmonary emphysema was detected. In one case (No. 207) in the left apical lobe of the lungs yellowish-grey foci were observed. In one animal (No. 035) in the medial lobe of the liver a 2-cm diameter yellowish-grey compact formation was found.

In the male **150 mg/kg** dose of the test item Dotated Potassium Humate Powder in two cases (No. 171 and No. 228) pulmonary emphysema occurred. In one animal (No. 222) sporadic miliary yellowish-brown foci were detected in the liver. In one case (No. 050) on the medial lobe of the liver (on nut-sized area) greyish-red coloured and nutmeg-like pattern was noticed.

In the recovery male **150 mg/kg** dose of the test item Dotated Potassium Humate Powder in one animal (No. 159) pulmonary emphysema was observed.

See Appendix 1.8 (page 1-2) and 2.8 (page 1-3)

In the female **control** group in two cases (No. 866 and No. 904) pulmonary emphysema was observed. In one animal (No. 891) the lobes of lungs were compact and yellowish-grey coloured. In one case (No. 866) circumscribed peritonitis was found (adhesion was detected between the liver and the diaphragm).

In the female **recovery control** group in one case (No. 864) pulmonary emphysema was found.

In the female **15 mg/kg** dose of the test item Dotated Potassium Humate Powder in three cases (No. 825, No. 828 and No. 926) pulmonary emphysema occurred. In one animal (No. 875) sporadic miliary yellowish-brown foci were observed.

In the female **50 mg/kg** dose of the test item Dotated Potassium Humate Powder in one case (No. 725) pulmonary emphysema occurred. In one animal (No. 889) bean-sized, white tumour-like formation was observed in the gallbladder. In this animal one-side pyelectasis was found and one of kidneys was smaller than normal.

In the female **150 mg/kg** dose of the test item Dotated Potassium Humate Powder in two cases (No. 859 and No. 860) pulmonary emphysema was detected. In one animals (No. 880) sporadic miliary yellowish-brown foci were found.

In the female **recovery 150 mg/kg** dose of the test item Dotated Potassium Humate Powder in one animal (No. 911) pulmonary emphysema was observed. In one case (No. 718) pinprick-sized haemorrhages were observed in the lungs.

See Appendix 1.8 (page 1-2) and 2.8 (page 4-6)

6.10. ORGAN WEIGHT DATA

Males

The organ weight data of the kidneys referring to the body weight data were statistically significantly lower in the low and middle dose groups than in the control group. The body weight referring organ weight values of the testes were statistically significantly higher in the high dose group than in the control group.
In the recovery males the brain referring organ weight of the lungs and of the kidneys were statistically significantly lower in the high dose group than in the control group.

See Appendix 1.9 (page 1-6) and 2.9 (page 1-6)

Females

In the low dose group the organ weight of the spleen, the body weight and the brain referring organ weight of it was significantly higher than in the control group. The organ weight of the spleen in the low dose group was double than in the other groups. The histopathological evaluation of the spleen in the low dose group did not show any pathological finding therefore these increased values of the spleen were in relation with the exsanquination process.

See Appendix 1.9 (page 7-12) and 2.9 (page 7-12)

6.11. HISTOPATHOLOGY AND HISTOMETRY

In the male **control** group in 2 cases zonal fatty infiltration (No. 214 and No. 163), in 4 cases hyaline deposits (No. 885, No. 214, No. 209 and No. 163) and in 1 case calcium deposits (No. 214) were detected in the kidneys. In 2 cases alveolar emphysema (No. 885 and No. 209) was observed in the lungs and in 2 cases involution (No. 885 and No. 209) was seen in the thymus.

In the recovery male **control** group in 1 case zonal fatty infiltration (No. 066) was detected in the kidney. In 2 cases alveolar emphysema (No. 066 and No. 227) was observed in the lungs. In 1 case cyst (No. 066) occurred in the pituitary.

In the male **15 mg/kg** dose of the test item Dotated Potassium Humate Powder in 1 case zonal fatty infiltration (No. 212), in 3 cases hyaline deposits (No. 226, No. 212 and No. 160) and in 2 cases calcium deposits (No. 212 and No. 160) were detectable in the kidneys. In 1 case alveolar emphysema and subacute catarrhal pneumonia (No. 212) were observed in the lungs. In 1 case involution (No. 160) was seen in the thymus and in 1 case cyst (No. 212) occurred in the pituitary.

In the male **50 mg/kg** dose of the test item Dotated Potassium Humate Powder in 1 case focal coagulative necrosis (No. 035) was established in the liver. In 1 case zonal fatty infiltration (No. 215), in 3 cases hyaline deposits (No. 826, No. 215 and No. 207), and in 1 case calcium deposits (No. 207) were detected in the kidneys. In 3 cases alveolar emphysema (No. 826, No. 215 and No. 035), in 1 case subacute catarrhal pneumonia (No. 207) were observed in the lungs. In 2 cases involution (No. 826 and No. 207) was seen in the thymus, in 1 case cyst occurred in the pituitary and in 1 case acute haemorrhage (No. 826) was detected in the heart.

In the male **150 mg/kg** dose of the test item Dotated Potassium Humate Powder in 3 cases centrolobular fine droplet fatty infiltration (No. 228, No. 171, No. 050) and in 1 case zonal decrease of glycogen content (No. 050) was established in the liver. In 1 case multifocal lympho-histiocytic infiltration (No. 050) occurred in the thyroid gland. In 1 case zonal fatty infiltration (No. 222), in 3 cases fine droplet diffuse fatty infiltration (No. 228, No. 171, No. 050), in 4 cases hyaline deposits (No. 228, No. 222, No. 171 and No. 050) and in 1 case calcium deposits (No. 050) were seen in the kidneys. In 2 cases alveolar emphysema (No. 228 and No. 171), was detected in the lungs. In 1 case involution (No. 222) was observed in the thymus. In 2 cases cyst (No. 222 and No. 171) occurred in the pituitary.

In the recovery male **150 mg/kg** dose of the test item Dotated Potassium Humate Powder in 2 cases hyaline deposits (No. 159 and No. 225) were observed in the kidneys. In 1 case alveolar emphysema (No. 159) was detected in the lungs. In 2 cases involution (No. 159 and No. 225) was seen in the thymus.

See Appendix 1.10 (page 1-6) and 2.10 (page 1-6)

In the female **control** group in 4 cases zonal fatty infiltration (No. 904, No. 891, No. 866 and No. 858), in 3 cases hyaline deposits (No. 904, No. 891 and No. 866) and in 2 cases calcium deposits (No. 904, No. 858) were detected in the kidneys. In 2 cases alveolar emphysema (No. 904 and No. 866), in 1 case catarrhal pneumonia (No. 891) was observed in the lungs. In 4 cases involution (No. 904, No. 891, No. 866 and No. 858) was seen in the thymus and in 1 case cyst (No. 866) occurred in the pituitary.

In the recovery female **control** group in 2 cases hyaline deposits and calcium deposits (No. 864 and No. 907) were observed in the kidneys. In 1 case alveolar emphysema (No. 907) occurred in the lungs.

In the female **15 mg/kg** dose of the test item Dotated Potassium Humate Powder in 4 cases zonal fatty infiltration (No. 926, No. 875, No. 828 and No. 825), in 3 cases hyaline deposits (No. 926, No. 828 and No. 825) and in 1 case calcium deposits (No. 825) were detected in the kidneys. In 4 cases alveolar emphysema (No. 926, No. 875, No. 828 and No. 825) was observed in the lung. In 3 cases involution (No. 926, No. 875 No. 825) was seen in the thymus and in 2 cases cyst (No. 875 and No. 825) occurred in the pituitary.

In the female **50 mg/kg** dose of the test item Dotated Potassium Humate Powder in 4 cases zonal fatty infiltration (No. 924, No. 889, No. 834 and No. 725), in 2 cases hyaline deposits (No. 889 and No. 834) were detected in the kidneys. In 2 cases alveolar emphysema (No. 924 and No. 725) was observed in the lungs. In 3 cases involution (No. 889, No. 834 and No. 725) was seen in the thymus. In 1 case cyst (No. 834) occurred in the pituitary.

In the female **150 mg/kg** dose of the test item Dotated Potassium Humate Powder In 2 cases centrolobular fine droplet fatty infiltration (slight degree) and in 3 cases zonal decrease in glycogen content (No. 890, No. 860 and No. 859) was detected in the liver. In 4 cases zonal fatty infiltration (No. 880, No. 890, No. 860 and No. 859), in 3 cases slight diffuse fatty infiltration (No. 880, No. 890 and No. 859), in 4 cases hyaline deposits (No. 880, No. 890, No. 860 and No. 859), and in 1 case calcium deposits (No. 859) were observed in the kidneys. In 3 cases alveolar emphysema (No. 880, No. 890 and No. 859) was established in the lungs. In 3 cases involution (No. 880, No. 860 and No. 859) was seen in the thymus. In 1 case acute focal myocardial necrosis (No. 880) and in 1 case subacute focal myocardial necrosis with restorative lesions (No. 860) occurred in the heart. In 2 cases cyst (No. 880 and No. 890) was detected in the pituitary

In the recovery female **150 mg/kg** dose of the test item Dotated Potassium Humate Powder in 2 cases zonal fatty infiltration (No. 718 and No.911), in 2 cases hyaline deposits (No. 718 and No. 911) and in 1 case calcium deposits (No. 911) were detected in the kidneys. In 1 case alveolar emphysema (No. 911) was observed in the lungs. In 2 cases involution (No. 718 and No.911) was seen in the thymus. In 1 case lympho-histiocytic inflammation (No. 911) was established in the thyroid gland. In 1 case cyst (No. 911) occurred in the pituitary.

See Appendix 1.10 (page 7-12) and 2.10 (7-12)

With the **histometrical examination** of the lymphoid organs neither hyperplastic nor regressive alterations were detected in any dose groups, referring to an immunostimulating or immunsuppressive effect of the test item.
The slight alterations observed in various organs and in different dose groups, in most cases showed no dose dependency and appeared only sporadically among the numerous measured objects.
The detected differences between the measured data of the various organs and dose groups must be in connection with the individual physiological differences. The histometrical data are summarised in the Appendix 1.12 (page1-2).

6.12. BONE MARROW SMEAR EVALUATION

In the male **control** group 3 animals had normal haemopoiesis and one animal (No. 214) had a slight increase in erythropoiesis.

In the male **recovery control** group both animals had normal haemopoiesis.

In the male **15 mg/kg** dose of the test item Dotated Potassium Humate Powder all animals had normal haemopoiesis.

In the male **50 mg/kg** dose of the test item Dotated Potassium Humate Powder three animals had normal haemopoiesis, one animal (No. 215) had minimal increase in erythropoiesis.

In the male **150 mg/kg** dose of the test item Dotated Potassium Humate Powder three animals had normal haemopoiesis, one animal (No. 050) had a slight increase in erythropoiesis.

In the recovery male **150 mg/kg** dose of the test item Dotated Potassium Humate Powder both animals had normal haemopoiesis.

See Appendix 1.11 (page 1-3) and 2.11 (page 1-3)

In the female **control** group three animals had normal haemopoiesis, one animal (No. 866) had a slight increase in erythropoiesis.

In the female **recovery control** group one animal had normal haemopoiesis, the other animal (No. 718) had minimal increase in erythropoiesis.

In the female **15 mg/kg** dose of the test item Dotated Potassium Humate Powder all animals had normal haemopoiesis.

In the female **50 mg/kg** dose of the test item Dotated Potassium Humate Powder the haemopoiesis was normal in all animals.

In the female **150 mg/kg** dose of the test item Dotated Potassium Humate Powder three animals had normal haemopoiesis, one animal (No. 859) had a slight increase in erythropoiesis.

In the female **recovery 150 mg/kg** dose of the test item Dotated Potassium Humate Powder one animal had normal haemopoiesis. from the other animal (No. 907) the slide was not evaluated due to technical failure.

See Appendix 1.11 (page 3-6) and 2.11 (page 3-6)

7. DISCUSSION

In our experimental conditions the following clinical signs were observed:

- in control in one case diarrhea,
- in the 15 mg/kg dose groups in 3 cases vomiting and in 2 cases thin faeces,
- in the 50 mg/kg dose groups in 9 cases vomiting and 8 cases thin faeces,
- in the 150 mg/kg caused 127 cases vomiting and in 51 cases diarrhea or thin faeces.

The aetiology of the vomiting could be multifactorial. In apparition of this alteration played role the test item, but the role of the coprophagy and of other factors (psychological or pathological factors) cannot be excluded.
In the increased frequency of the thin, diarrheic faeces the test item effect cannot be excluded.
After 180 days of treatment statistically significant deviation in the body weight gain and food consumption data was not found.
The applied dose levels of Dotated Potassium Humate Powder did not cause any severe changes of the hematological and clinical chemical parameters during the 180-day oral administration, which could refer to the injury of any organs of vital importance.
The test item had a slight suspected, not proved, protein, albumin and cholesterol decreasing effect in both sexes mostly in the high dose groups and a slight calcium decreasing effect in the females of the high dose group. The decreased values remained between the physiological ranges.
All changes in urine parameters were within the physiological range and cannot be related to the test item effect.
No direct treatment related ECG alterations were found.
The observed alterations (electrical alternans, ST elevation, sinus arrest, right deviation, etc) occurred incidentally as individual findings and indicate alterations in stimulus generation and repolarization. In the appearance of these alterations the test item effect could be excluded.
No treatment related ophthalmological alterations were found during the study. The ball-like filiform formation in one side lens of one control animal was an individual alteration without toxicological meaning.
The pulmonary emphysema and the pinprick-sized haemorrhages in the lungs are alterations, which can be in connection with extermination and agony.
Macroscopic alteration in direct connection with the toxic effect of the test item was not found.
In the males the organ weight data of the kidneys referring to the body weight data were statistically significantly lower in the low and middle dose group dose group than in the control group. The body weight referring organ weight values of the testes were statistically significantly higher in the high dose group than in the control group. The histopathological examination was negative in case of the male genital organs, therefore the increased testes' weight values had only statistical, but no biological importance.
In the recovery males the brain referring organ weight of the lungs and of the kidneys were statistically significantly lower in the high dose group than in the control group.

In the females the organ weight of the spleen, the body weight and the brain referring organ weight of it were significantly higher in the low dose group than in the control group. The organ weight of the spleen in the low dose group was double than in the other groups. The histopathological evaluation of the spleen in the low dose group did not show any pathological finding therefore these increased values of the spleen were in relation with the exsanquination process.
The centrolobular, slight, fine droplet fatty infiltration in the liver and the diffuse slight fine droplet fatty infiltration in the kidneys occurred only in the 150 mg/kg male and female groups (liver: 228, 171, 050, 860, 859, kidneys: 228, 171, 050, 880, 890, 859) so, these findings seems to be treatment related lesions. The slight diffuse fatty infiltration in the liver and kidneys were not detectable in the organs of recovery animals, so, it had a reversible character.
The zonal decrease in glycogen content of hepatocytes in some animals belonging to the 150 mg/kg dose groups (No. 050, No. 890, No. 860, No. 859) seems to be the consequence of the metabolic charging effect of the high dose of test item.
Acute focal myocardial necrosis and subacute focal myocardial necrosis with restorative lesions occurred in one female animal each (No. 880, No. 860), in the 150 mg/kg dose group. These alterations macroscopically were not noticed.
However, these alterations could be developed as individual disorders in connection with local myocardial hypoxia because of stress or other origin, it can not be excluded that the long term rationing of the high dose of test item played role in the pathogenesis of these alterations.
The focal alveolar emphysema in the lungs and the acute haemorrhage in the heart - without muscle fibre damage - may be considered as consequence of hypoxia, dyspnoea and circulatory disturbance developed during exsanguination.
The involution of the thymus in this age is a physiologic process.
The zonal fatty infiltration, the calcium deposits and the hyaline deposits in the kidneys may be considered to be of nutrition-physiological-metabolic origin.
The subacute catarrhal pneumonia occurred sporadically in the lungs could be in connection with a local infection.
The focal coagulative necrosis in the liver, the cyst in the pituitary and the multifocal lympho-histiocytic infiltration in the thyroid gland (non-purulent thyroiditis) are individual disorders.
With the histometrical examination of the lymphoid organs neither hyperplastic nor regressive alterations were detected in any dose groups, referring to an immunostimulating or immunsuppressive effect of the test item.
The slight alterations observed in various organs and in different dose groups, in most cases showed no dose dependency and appeared only sporadically.
The detected differences between the measured data of the different organs and dose groups must be in connection with the individual physiological differences.
Based on the results of the histometrical examination no dose dependent or treatment related alterations could be detected which concerned more or all of the examined lymphoid organs of the experimental animals.
No morphological evidence of acute or subacute injury of the alimentary tract, the skeleton, the muscular system, or the central or peripheral nervous system was detected.

The structure and the cell morphology of the endocrine glands were the same at the control and treated animals.
It should be noted that the different size and weight of the uterus and ovaries and the individual difference in the development of secretory alveoli in the mammary glands at some female animals could be connected with the different neuro-hormonal status.
No morphological signs of neuro-hormonal disorder were detected in the treated female and male animals.
A slight increase in erythropoiesis within the physiological range was found in males and females, in control and treated groups, too. There were not seen any pathologic cells or pathologic alterations of normal cell distribution.
Under our experimental conditions the item Dotated Potassium Humate Powder did not influence the bone marrow function of the animals.

8. CONCLUSION

The test item marked Dotated Potassium Humate Powder administered orally to dogs in doses of 15, 50 and 150 mg/kg, respectively for 180 days caused dose in depending increased frequency of vomiting and thin faeces (diarrhea) in 50 and 150 mg/kg dose groups.
Histopathologically in our experimental conditions the test item Dotated Potassium Humate Powder caused slight degree reversible fine droplet centrolobular fatty infiltration of the liver in three male and two female animals in the 150 mg/kg dose groups, and fine droplet diffuse fatty infiltration in the kidneys in three female and three male animals in the 150 mg/kg dose groups. In addition, no provable, but can not be excluded that the long term rationing of the high dose of test item played role in the pathogenesis of acute focal myocardial necrosis in one female animal and of subacute focal myocardial necrosis in anther female animal.

Under the present experimental conditions the NOAEL -no observed adverse effect level- is 15 mg/kg/day.

APPENDICES

CLINICAL OBSERVATIONS SUMMARY INCIDENCIAL DATA

TEST ITEM: DOTATED POTASSIUM HUMATE POWDER TEST SYSTEM: BEAGLE DOG MODE OF APPLICATION: ORAL

STUDY CODE: 00/518-107K SEX: MALE

CLINICAL SIGNS	DOSAGE mg/kg	Placebo control		15		50		150	
		Σ	%	Σ	%	Σ	%	Σ	%
Vomiting		-	-	-	-	1	0,14	5	0,46
Vomiting (foamy content)		-	-	-	-	-	-	6	0,56
Vomiting (foamy, test item content)		-	-	-	-	1	0,14	12	1,11
Vomiting (test item content)		-	-	-	-	-	-	9	0,83
Vomiting (faeces content)		-	-	-	-	1	0,14	1	0,09
Vomiting before treatment		-	-	1	0,14	-	-	-	-
Thin faeces		-	-	-	-	1	0,14	11	1,02
Thin test item content faeces		-	-	-	-	-	-	1	0,09
Diarrhea		1	0,09	-	-	-	-	1	0,09
Thin faeces (before treatment)		-	-	2	0,28	1	0,14	9	0,83
Thin, test item content faeces before treatment		-	-	-	-	-	-	2	0,19
Costal arch pean and peanut-sized formation (left side)		-	-	70	9,72	-	-	-	-
Iliacal region alopecia (left side)		-	-	-	-	-	-	7	0,65
Abdominal region 3 cm diameter alopecia (left side)		-	-	-	-	-	-	30	2,78

CLINICAL OBSERVATIONS SUMMARY INCIDENCIAL DATA

TEST ITEM: DOTATED POTASSIUM HUMATE POWDER TEST SYSTEM: BEAGLE DOG MODE OF APPLICATION: ORAL

STUDY CODE: 00/518-107K SEX: FEMALE

CLINICAL SIGNS	DOSAGE mg/kg	Placebo control		15		50		150	
		Σ	%	Σ	%	Σ	%	Σ	%
Vomiting		-	-	-	-	-	-	8	0,74
Vomiting (foamy content)		-	-	-	-	4	0,56	41	3,8
Vomiting (test item content)		-	-	-	-	-	-	14	1,3
Vomiting (foamy, test item content)		-	-	1	0,14	2	0,28	20	1,85
Vomiting (faeces content)		-	-	-	-	-	-	8	0,74
Vomiting before treatment		-	-	1	0,14	-	-	2	0,19
Vomiting (foamy saliva content)		-	-	-	-	-	-	1	0,09
Salivation		-	-	-	-	-	-	1	0,09
Thin faeces		-	-	-	-	4	0,56	19	1,76
Thin, test item content faeces		-	-	-	-	-	-	1	0,09
Diarrhea		-	-	-	-	-	-	2	0,19
Thin faeces before treatment		-	-	-	-	2	0,28	7	0,65
Thin, test item content faeces before treatment		-	-	-	-	-	-	1	0,09
Kyphotic posture		-	-	-	-	-	-	1	0,09
Mamary gland (posterior, left side) nut-sized, dark formation with irregular surface		-	-	-	-	-	-	15	1,39

Macro-and microelement levels in blood of dogs participating in a 6 months repeated dose toxicology of Dotated Humifulvate

Statistical evaluation

In order to determine the element level in blood, the original protocol of 6 months repeated dose toxicology of Humifulvate in dogs, has been modified: blood samples were drawn from each animal belonging to the control group as well as from each animal belonging to the highest dose (150 mg/kg) treated group at the end of 1st, 3rd, and 6th month of treatment and analysed by ICP-OES method (Appendix I).
Because the treated group thus has no "before treatment" values, the actual levels were compared to those of the control group.

Detailed analysis

Although the test substance contains - rather low amount of - aluminium - originating from the peat - the blood **aluminium** level does not significantly differs from that of the control group.

There were no significant changes either in the treated or in the control group in blood **calcium** in spite of the very high dose applied.

No changes were observed in case of **cadmium** in the controll and treated group, though the highest determined value was lower in the treated group.

The levels of **cobalt, chromium and copper** have not changed. The **iron** levels of blood showed an increasing tendency in both groups, however the changes were not significant.

The **lithium** blood levels decreased in both groups therefore there were no significant differences between groups in the same treatment period.

An increasing tendency has been observed in the treated group in case of **manganese,** however, the difference was not significant.

As to the **molybdenum**, while in the treated group almost 90 % (32 out of 36) of the samples showed detectable levels, more than the half of the samples molybdenum level fell below the detection limit. However, comparing between the groups the actual, measured values, this difference turned not to be significant.

The amount of blood **nickel** did not changed in the control group, while in the control group an increasing tendency could be observed: within the treated group, an 1,5-fold increase could be seen at the end of the study, that is doublefold comparing to the control, though even this increase is not significant. The test substance itself does not contain added nickel (≤0,5 mg/kg).

Both in the treated and control groups, the **lead** blood levels decreased during the experiment: 0,3627→0,1634 mg/kg (control); 0,4575→0,238 mg/kg(treated), showing the same tendency.

The **selenium** blood levels showed significant increase in all of the treated groups compared to the control. At the end of the 1st and 3rd months the difference is strongly significant, the 6th month value is at the border of significance ($p = 0,09$).

The **vanadium** content of the blood showed similarity to that of the molybdenum. While in the control group 1/3 of the levels fell below the detection limit, every value was above in the treated group. In each time, the difference between the control and treated group was strongly significant.

The actual values as well as the statistical significances are shown in Appendix II.

The results hint at the absorption of the necessary trace elements (Mo, Se, V), while toxic accumulation as well as detectable "chelation out" effect of these elements have not been observed.

Dog	Trace element								
	Al	As	Ba	Be	Bi	Ca	Cd	Co	Cr
Control 066/1	0,16	0,18	0,05	nd	0,04	66,5	0,003	0,023	0,02
Control 163/1	0,19	0,17	0,07	nd	0,04	72,1	0,0041	0,019	0,009
Control 209/1	0,11	0,14	0,07	nd	0,053	64,8	0,0057	0,022	0,005
Control 214/1	0,16	0,15	0,062	nd	0,033	70,9	0,006	0,21	0,007
Control 227/1	0,15	0,13	0,063	nd	0,04	66,9	0,0064	0,022	0,003
Control 858/1	0,09	0,15	0,136	nd	0,05	60,9	0,005	0,019	0,002
Control 864/1	0,18	0,04	0,06	nd	0,065	58,9	0,0093	0,026	0,009
Control 866/1	0,12	0,06	0,055	nd	0,066	55,1	0,0081	0,027	0,002
Control 885/1	0,06	0,11	0,058	nd	0,033	65	0,0053	0,018	0,004
Control 891/1	0,12	0,12	0,056	nd	0,07	57,7	0,0066	0,025	0,0044
Control 904/1	0,09	0,07	0,044	nd	0,076	52,7	0,0085	0,03	0,002
Control 907/1	0,12	0,08	0,054	nd	0,054	62,7	0,0059	0,021	0,007
n	*12*	*12*	*12*	*0*	*12*	*12*	*12*	*12*	*12*
mean	*0,129*	*0,117*	*0,065*		*0,052*	*62,85*	*0,0062*	*0,039*	*0,0062*
SD	*0,0394*	*0,0452*	*0,0236*		*0,0149*	*6,012*	*0,0018*	*0,0541*	*0,00505*
median	*0,12*	*0,125*	*0,059*		*0,0515*	*63,75*	*0,00595*	*0,0225*	*0,0047*
min	*0,06*	*0,04*	*0,044*		*0,033*	*52,7*	*0,003*	*0,018*	*0,002*
max	*0,19*	*0,18*	*0,136*		*0,076*	*72,1*	*0,0093*	*0,21*	*0,02*
Control 066/2	0,245	0,08	0,08	nd	0,05	65,1	0,0038	0,0264	0,041
Control 163/2	0,07	0,17	0,102	nd	0,04	62,4	0,0032	0,02	0,005
Control 209/2	0,05	0,11	0,081	nd	0,051	62,5	0,0055	0,026	0,001
Control 214/2	0,09	0,07	0,073	nd	0,06	63,2	0,0066	0,024	0,009
Control 227/2	0,1	0,085	0,093	nd	0,06	58,9	0,0072	0,026	0,002
Control 858/2	0,13	0,07	0,096	nd	0,048	65,7	0,006	0,022	0,015
Control 864/2	0,12	0,02	0,107	nd	0,068	55,4	0,0089	0,023	0,032
Control 866/2	0,1	0,04	0,155	nd	0,052	56,6	0,0078	0,026	0,003
Control 891/2	0,13	0,07	0,092	nd	0,05	62,3	0,0067	0,026	0,0034
Control 885/2	0,09	0,2	0,081	nd	0,048	61,3	0,0074	0,024	0,01
Control 904/2	0,11	0,07	0,092	nd	0,059	52	0,0066	0,023	0,003
Control 907/2	0,34		0,292	nd	0,018	63	0,0068	0,018	0,19
n	*12*	*11*	*12*	*0*	*12*	*12*	*12*	*12*	*12*
mean	*0,131*	*0,090*	*0,112*		*0,050*	*60,70*	*0,0064*	*0,024*	*0,0262*
SD	*0,0814*	*0,0529*	*0,0605*		*0,0126*	*4,143*	*0,0016*	*0,0027*	*0,05312*
median	*0,105*	*0,07*	*0,0925*		*0,0505*	*62,35*	*0,00665*	*0,024*	*0,007*
min	*0,05*	*0,02*	*0,073*		*0,018*	*52*	*0,0032*	*0,018*	*0,001*
max	*0,34*	*0,2*	*0,292*		*0,068*	*65,7*	*0,0089*	*0,0264*	*0,19*
Control 066/3	0,08	0,07	0,064	nd	0,04	61,2	0,0049	0,026	0,002
Control 163/3	0,08	0,08	0,102	nd	0,05	61	0,0051	0,024	0,002
Control 209/3	0,11	0,07	0,09	nd	0,068	60,7	0,0073	0,027	0,006
Control 214/3	0,11	0,06	0,106	nd	0,045	65	0,007	0,024	0,003
Control 227/3	0,15	0,09	0,085	nd	0,06	65,2	0,0068	0,024	0,023
Control 858/3	0,08	0,12	0,091	nd	0,062	57,5	0,0066	0,025	0,002
Control 864/3	0,1	0,04	0,091	nd	0,053	75	0,0069	0,0218	0,006
Control 866/3	0,1	0,07	0,111	nd	0,066	54,8	0,007	0,03	0,004
Control 885/3	0,13	0,14	0,108	nd	0,055	61,4	0,0069	0,023	0,003
Control 891/3	0,11	0,06	0,075	nd	0,055	58,8	0,0067	0,024	0,002
Control 904/3	0,11	0,05	0,2	nd	0,062	55,8	0,0079	0,026	0,016
Control 907/3	0,12	0,065	0,077	nd	0,048	69,7	0,0073	0,024	0,0042
n	*12*	*12*	*12*	*0*	*12*	*12*	*12*	*12*	*12*
mean	*0,107*	*0,076*	*0,100*		*0,055*	*62,18*	*0,0067*	*0,025*	*0,0061*
SD	*0,0210*	*0,0285*	*0,0346*		*0,0086*	*5,812*	*0,0009*	*0,0021*	*0,00659*
median	*0,11*	*0,07*	*0,091*		*0,055*	*61,1*	*0,0069*	*0,024*	*0,0035*
min	*0,08*	*0,04*	*0,064*		*0,04*	*54,8*	*0,0049*	*0,0218*	*0,002*
max	*0,15*	*0,14*	*0,2*		*0,068*	*75*	*0,0079*	*0,03*	*0,023*

Dog

	Cu	Fe	Li	Mg	Mn	Mo	Ni	P	Pb
Control 066/1	0,715	412	0,008	35,9	0,027	0,005	0,071	498	0,119
Control 163/1	0,547	368	0,0063	34,5	0,0243	0,007	0,096	492	0,432
Control 209/1	0,537	525	0,0068	39,1	0,03	0,004	0,03	559	0,562
Control 214/1	0,556	460	0,006	36,4	0,027	0,004	0,082	503	0,298
Control 227/1	0,601	520	0,0067	36,2	0,031	0,003	0,046	526	0,283
Control 858/1	0,576	448	0,016	34,7	0,03	nd	0,018	464	0,104
Control 864/1	0,666	694	0,0041	38,6	0,05	nd	0,023	560	0,261
Control 866/1	0,625	703	0,0058	40,9	0,036	nd	0,037	567	1,1
Control 885/1	0,651	408	0,0072	35,9	0,026	0,003	0,016	467	0,267
Control 891/1	0,609	618	0,0075	44,1	0,038	nd	0,071	582	0,344
Control 904/1	0,613	815	0,0075	45,1	0,068	nd	0,02	649	0,403
Control 907/1	0,566	509	0,0069	34,4	0,041	nd	0,03	504	0,179
n	*12*	*12*	*12*	*12*	*12*	*6*	*12*	*12*	*12*
mean	*0,60517*	*540,0*	*0,0074*	*37,98*	*0,03569*	*0,0043*	*0,045*	*530,9*	*0,3627*
SD	*0,05324*	*138,77*	*0,00289*	*3,672*	*0,01259*	*0,00151*	*0,02782*	*54,09*	*0,26632*
median	*0,605*	*514,5*	*0,00685*	*36,3*	*0,0305*	*0,004*	*0,0335*	*515*	*0,2905*
min	*0,537*	*368*	*0,0041*	*34,4*	*0,0243*	*0,003*	*0,016*	*464*	*0,104*
max	*0,715*	*815*	*0,016*	*45,1*	*0,068*	*0,007*	*0,096*	*649*	*1,1*
Control 066/2	0,66	528	0,0015	36,6	0,02	0,05	0,064	506	0,072
Control 163/2	0,603	439	0,0015	36,9	0,017	0,004	0,014	463	0,064
Control 209/2	0,562	604	0,0012	40,6	0,024	0,003	0,018	602	0,158
Control 214/2	0,57	613	0,0013	40,5	0,026	nd	0,033	563	0,087
Control 227/2	0,631	591	0,0015	37,1	0,024	nd	0,021	510	0,2
Control 858/2	0,587	497	0,0016	35,7	0,036	0,004	0,039	480	0,554
Control 864/2	0,631	662	0,0014	38,3	0,044	nd	0,119	513	0,187
Control 866/2	0,558	697	0,0013	41,8	0,027	nd	0,027	570	0,184
Control 891/2	0,569	586	0,0013	40,9	0,029	nd	0,069	557	0,591
Control 885/2	0,642	582	0,0016	38,5	0,025	0,008	0,028	477	0,351
Control 904/2	0,593	562	0,0016	38,2	0,042	nd	0,035	494	0,075
Control 907/2	0,611	549	0,0047	37,3	0,069	0,02	0,115	505	0,108
n	*12*	*12*	*12*	*12*	*12*	*6*	*12*	*12*	*12*
mean	*0,60142*	*575,8*	*0,00171*	*38,53*	*0,03192*	*0,0148*	*0,0485*	*520,0*	*0,2193*
SD	*0,03395*	*69,28*	*0,00095*	*1,968*	*0,01429*	*0,01836*	*0,03609*	*43,05*	*0,18352*
median	*0,598*	*584*	*0,0015*	*38,25*	*0,0265*	*0,006*	*0,034*	*508*	*0,171*
min	*0,558*	*439*	*0,0012*	*35,7*	*0,017*	*0,003*	*0,014*	*463*	*0,064*
max	*0,66*	*697*	*0,0047*	*41,8*	*0,069*	*0,05*	*0,119*	*602*	*0,591*
Control 066/3	0,627	569	0,0013	35,8	0,017	0,006	0,028	484	0,078
Control 163/3	0,556	560	0,0014	39	0,0186	0,003	0,0278	520	0,068
Control 209/3	0,572	622	0,0012	39,5	0,027	nd	0,032	548	0,808
Control 214/3	0,579	546	0,0011	39,2	0,03	nd	0,037	501	0,069
Control 227/3	0,593	590	0,00134	35,1	0,025	0,004	0,052	481	0,252
Control 858/3	0,637	596	0,0012	38,9	0,033	nd	0,021	533	0,088
Control 864/3	0,633	543	0,0018	36,5	0,04	0,017	0,073	534	0,075
Control 866/3	0,576	750	0,0013	43,1	0,049	nd	0,022	581	0,137
Control 885/3	0,593	564	0,0013	35,4	0,025	nd	0,062	471	0,0736
Control 891/3	0,638	602	0,0013	43,1	0,0297	nd	0,03	539	0,114
Control 904/3	0,59	677	0,0012	38,2	0,053	nd	0,031	545	0,107
Control 907/3	0,58	574	0,0018	37,5	0,034	0,008	0,091	511	0,091
n	*12*	*12*	*12*	*12*	*12*	*5*	*12*	*12*	*12*
mean	*0,59783*	*599,4*	*0,00135*	*38,44*	*0,03178*	*0,0076*	*0,04223*	*520,7*	*0,1634*
SD	*0,02846*	*60,10*	*0,00022*	*2,658*	*0,01102*	*0,00559*	*0,02234*	*32,35*	*0,20928*
median	*0,5915*	*582*	*0,0013*	*38,55*	*0,02985*	*0,006*	*0,0315*	*526,5*	*0,0895*
min	*0,556*	*543*	*0,0011*	*35,1*	*0,017*	*0,003*	*0,021*	*471*	*0,068*
max	*0,638*	*750*	*0,0018*	*43,1*	*0,053*	*0,017*	*0,091*	*581*	*0,808*

Dog

	Sb	Se	Si	Sn	Sr	Te	Ti	Tl	V
Control 066/1	0,02	0,284058	0,31746	0,055	0,05	nd	nd	0,0096	nd
Control 163/1	nd	0,268841	0,211111	0,311	0,046	nd	nd	0,0071	0,0013
Control 209/1	0,2	0,306522	0,174603	0,28	0,039	nd	nd	0,005	0,0007
Control 214/1	nd	0,244203	0,412698	0,054	0,041	nd	nd	0,0056	0,0005
Control 227/1	0,02	0,266667	1,698413	0,12	0,04	nd	nd	0,0095	nd
Control 858/1	0,019	0,297826	0,029365	0,04	0,039	nd	nd	0,0062	nd
Control 864/1	0,025	0,312319	0,162698	0,124	0,032	nd	nd	0,005	0,0015
Control 866/1	0,024	0,306522	0,139683	0,18	0,03	nd	nd	0,0046	nd
Control 885/1	0,02	0,236957	0,115079	0,084	0,05	nd	nd	0,004	nd
Control 891/1	0,023	0,307971	0,160317	0,18	0,032	nd	nd	0,0082	nd
Control 904/1	0,031	0,353623	0,150794	0,12	0,04	nd	nd	0,004	0,0006
Control 907/1	0,02	0,288406	0,135714	0,056	0,047	nd	nd	0,0055	0,0006
n	*10*	*12*	*12*	*12*	*12*	*0*	*0*	*12*	*6*
mean	*0,0402*	*0,28949*	*0,30899*	*0,13367*	*0,0405*			*0,00619*	*0,00087*
SD	*0,05626*	*0,03221*	*0,44843*	*0,08915*	*0,00683*			*0,00198*	*0,00042*
median	*0,0215*	*0,29312*	*0,16151*	*0,12*	*0,04*			*0,00555*	*0,00065*
min	*0,019*	*0,23696*	*0,02937*	*0,04*	*0,03*			*0,004*	*0,0005*
max	*0,2*	*0,35362*	*1,69841*	*0,311*	*0,05*			*0,0096*	*0,0015*
Control 066/2	0,02	0,322464	0,52381	0,04	0,048	nd	nd	0,0088	nd
Control 163/2	nd	0,255797	0,265873	0,02	0,042	nd	nd	0,0051	nd
Control 209/2	0,02	0,356522	0,278571	0,031	0,047	nd	nd	0,0028	nd
Control 214/2	0,02	0,25	0,209524	0,035	0,044	nd	nd	0,0096	0,0005
Control 227/2	0,02	0,32029	0,311111	0,07	0,038	nd	nd	0,0045	0,0005
Control 858/2	0,02	0,296377	0,322222	0,045	0,041	nd	nd	0,0064	0,0008
Control 864/2	0,025	0,302899	0,561111	0,046	0,031	nd	nd	0,0075	0,001
Control 866/2	0,026	0,32971	0,387302	0,04	0,033	nd	nd	0,0065	0,0006
Control 891/2	0,02	0,345652	0,469048	0,05	0,037	nd	nd	0,0078	0,0008
Control 885/2	0,02	0,289855	0,312698	0,04	0,042	nd	nd	0,006	nd
Control 904/2	0,02	0,310145	0,287302	0,04	0,044	nd	nd	0,0069	0,0012
Control 907/2	0,024	0,25942	0	0,06	0,078	nd	nd	0,021	
n	*11*	*12*	*12*	*12*	*12*	*0*	*0*	*12*	*7*
mean	*0,02136*	*0,30326*	*0,32738*	*0,04308*	*0,04375*			*0,00774*	*0,00077*
SD	*0,00238*	*0,03474*	*0,14961*	*0,01298*	*0,01196*			*0,00457*	*0,00026*
median	*0,02*	*0,30652*	*0,3119*	*0,04*	*0,042*			*0,0067*	*0,0008*
min	*0,02*	*0,25*	*0*	*0,02*	*0,031*			*0,0028*	*0,0005*
max	*0,026*	*0,35652*	*0,56111*	*0,07*	*0,078*			*0,021*	*0,0012*
Control 066/3	0,02	0,328986	0,103175	0,032	0,039	nd	nd	0,0034	nd
Control 163/3	0,023	0,278261	0,190476	0,028	0,037	nd	nd	0,005	0,0005
Control 209/3	0,02	0,342029	0,380952	0,057	0,035	nd	nd	0,005	0,0007
Control 214/3	0,02	0,254348	0,325397	0,026	0,035	nd	nd	0,008	0,0005
Control 227/3	0,02	0,363043	0,280952	0,065	0,04	nd	nd	0,0067	0,0007
Control 858/3	0,02	0,323188	0,27619	0,047	0,028	nd	nd	0,0041	0,0008
Control 864/3	0,02	0,344928	0,62381	0,027	0,067	nd	nd	0,0053	0,001
Control 866/3	0,028	0,342754	0,219048	0,042	0,033	nd	nd	0,005	0,0006
Control 885/3	0,02	0,315217	0,25873	0,032	0,04	nd	nd	0,0065	nd
Control 891/3	0,021	0,305072	0,896825	0,03	0,035	nd	nd	0,007	0,0011
Control 904/3	0,03	0,315942	0,342857	0,03	0,03	nd	nd	0,006	0,0011
Control 907/3	0,026	0,30942	0,636508	0,035	0,064	nd	nd	0,006	0,0008
n	*12*	*12*	*12*	*12*	*12*	*0*	*0*	*12*	*10*
mean	*0,02233*	*0,3186*	*0,37791*	*0,03758*	*0,04025*			*0,00567*	*0,00078*
SD	*0,00363*	*0,03011*	*0,22772*	*0,01265*	*0,01237*			*0,00129*	*0,00023*
median	*0,02*	*0,31957*	*0,30317*	*0,032*	*0,036*			*0,00565*	*0,00075*
min	*0,02*	*0,25435*	*0,10317*	*0,026*	*0,028*			*0,0034*	*0,0005*
max	*0,03*	*0,36304*	*0,89683*	*0,065*	*0,067*			*0,008*	*0,0011*

Dog

	Zn	Se	V
Control 066/1	2,957265	0,284058	nd
Control 163/1	2,9401709	0,2688406	0,0013
Control 209/1	3	0,3065217	0,0007
Control 214/1	3,1196581	0,2442029	0,0005
Control 227/1	2,9487179	0,2666667	nd
Control 858/1	3,1282051	0,2978261	nd
Control 864/1	3,6324786	0,3123188	0,0015
Control 866/1	4,2735043	0,3065217	nd
Control 885/1	3,025641	0,2369565	nd
Control 891/1	3,8717949	0,307971	nd
Control 904/1	4,9145299	0,3536232	0,0006
Control 907/1	3,0683761	0,2884058	0,0006
n	*12*	0,3224638	nd
mean	*3,4067*	0,2557971	nd
SD	*0,6401*	0,3565217	nd
median	*3,0940171*	0,25	0,0005
min	*2,9401709*	0,3202899	0,0005
max	*4,9145299*	0,2963768	0,0008
Control 066/2	2,8376068	0,3028986	0,001
Control 163/2	2,991453	0,3297101	0,0006
Control 209/2	3,1794872	0,3456522	0,0008
Control 214/2	3,6410256	0,2898551	nd
Control 227/2	3,1880342	0,3101449	0,0012
Control 858/2	3,3162393	0,2594203	
Control 864/2	3,2820513	0,3289855	nd
Control 866/2	4,0854701	0,2782609	0,0005
Control 891/2	3,6068376	0,342029	0,0007
Control 885/2	3,7094017	0,2543478	0,0005
Control 904/2	3,5128205	0,3630435	0,0007
Control 907/2	3,2307692	0,3231884	0,0008
n	*12*	0,3449275	0,001
mean	*3,3818*	0,3427536	0,0006
SD	*0,3440*	0,3152174	nd
median	*3,2991453*	0,3050725	0,0011
min	*2,8376068*	0,315942	0,0011
max	*4,0854701*	0,3094203	0,0008
Control 066/3	2,9145299		
Control 163/3	3,4102564	0,0941773	0,090159733
Control 209/3	3,3589744	*0,0913649*	*0,111107379*
Control 214/3	3,5897436	*0,0616765*	*0,111088972*
Control 227/3	3,4102564		
Control 858/3	3,5384615		
Control 864/3	3,1025641		
Control 866/3	4,1794872		
Control 885/3	3,5470085		
Control 891/3	3,6581197		
Control 904/3	3,8803419		
Control 907/3	3,2136752		
n	*12*		
mean	*3,4836*		
SD	*0,3383*		
median	*3,474359*		
min	*2,9145299*		
max	*4,1794872*		

	Al	As	Ba	Be	Bi	Ca	Cd	Co	Cr
150 mg 050/1	0,17	0,1	0,087	nd	0,06	61,7	0,0061	0,026	0,008
150 mg 159/1	0,06	0,09	0,058	nd	0,055	60,3	0,0072	0,024	0,007
150 mg 171/1	0,33	0,11	0,11	nd	0,052	72,9	0,0126	0,025	0,028
150 mg 222/1	0,12	0,13	0,09	nd	0,038	67,8	0,0059	0,025	0,017
150 mg 225/1	0,19	0,15	0,067	nd	0,08	56,9	0,0079	0,028	0,009
150 mg 228/1	0,15	0,14	0,071	nd	0,052	68,9	0,0059	0,025	0,005
150 mg 718/1	0,12	0,076	0,062	nd	0,056	58,6	0,0061	0,026	0,001
150 mg 859/1	0,5	0,13	0,108	nd	0,055	56,5	0,0058	0,025	0,007
150 mg 860/1	0,14	0,04	0,069	nd	0,05	59,8	0,0059	0,024	0,332
150 mg 880/1	0,08	0,04	0,101	nd	0,05	61,5	0,0049	0,021	0,002
150 mg 890/1	0,18	0,056	0,075	nd	0,066	67,4	0,008	0,028	0,004
150 mg 911/1	0,13	0,06	0,071	nd	0,055	60,4	0,0063	0,024	0,003
n	*12*	*12*	*12*	*0*	*12*	*12*	*12*	*12*	*12*
mean	*0,181*	*0,094*	*0,081*		*0,056*	*62,73*	*0,0069*	*0,025*	*0,03525*
SD	*0,1212*	*0,0392*	*0,0180*		*0,0101*	*5,234*	*0,0020*	*0,0019*	*0,09375*
median	*0,145*	*0,095*	*0,073*		*0,055*	*60,95*	*0,0061*	*0,025*	*0,007*
min	*0,06*	*0,04*	*0,058*		*0,038*	*56,5*	*0,0049*	*0,021*	*0,001*
max	*0,5*	*0,15*	*0,11*		*0,08*	*72,9*	*0,0126*	*0,028*	*0,332*
150 mg 050/2	0,11	0,03	0,151	nd	0,058	57,9	0,0075	0,026	0,011
150 mg 159/2	0,06	0,05	0,096	nd	0,065	58,5	0,0067	0,027	0,002
150 mg 171/2	0,12	0,06	0,133	nd	0,06	56,7	0,0072	0,026	0,011
150 mg 222/2	0,13	0,11	0,11	nd	0,046	69,2	0,0052	0,024	0,025
150 mg 225/2	0,32	0,12	0,103	nd	0,039	58,7	0,0069	0,025	0,005
150 mg 228/2	0,08	0,148	0,096	nd	0,048	63,9	0,0049	0,025	0,003
150 mg 718/2	0,1	0,08	0,099	nd	0,04	63,9	0,0064	0,022	0,05
150 mg 859/2	0,1	0,05	0,078	nd	0,045	65,7	0,0051	0,021	0,003
150 mg 860/2	0,16	0,03	0,121	nd	0,044	54,5	0,006	0,02	0,008
150 mg 880/2	0,14	0,03	0,118	nd	0,065	59,4	0,0067	0,027	0,004
150 mg 890/2	0,11	0,04	0,123	nd	0,056	61,9	0,0056	0,025	0,003
150 mg 911/2	0,09	0,02	0,105	nd	0,065	50,1	0,0082	0,03	0,002
n	*12*	*12*	*12*	*0*	*12*	*12*	*12*	*12*	*12*
mean	*0,127*	*0,064*	*0,111*		*0,053*	*60,03*	*0,0064*	*0,025*	*0,01058*
SD	*0,0665*	*0,0415*	*0,0194*		*0,0100*	*5,214*	*0,0010*	*0,0028*	*0,01402*
median	*0,11*	*0,05*	*0,1075*		*0,052*	*59,05*	*0,00655*	*0,025*	*0,0045*
min	*0,06*	*0,02*	*0,078*		*0,039*	*50,1*	*0,0049*	*0,02*	*0,002*
max	*0,32*	*0,148*	*0,151*		*0,065*	*69,2*	*0,0082*	*0,03*	*0,05*
150 mg 050/3	0,2	0,06	0,11	nd	0,06	55,1	0,0073	0,027	0,011
150 mg 159/3	0,09	0,08	0,085	nd	0,048	56,8	0,0059	0,024	0,004
150 mg 171/3	0,05	0,07	0,168	nd	0,056	65,1	0,0057	0,023	0,005
150 mg 222/3	0,16	0,23	0,25	nd	0,05	61,7	0,0071	0,027	0,009
150 mg 225/3	0,1	0,095	0,106	nd	0,059	54,1	0,0071	0,026	0,008
150 mg 228/3	0,05	0,12	0,1	nd	0,04	60,8	0,0057	0,028	0,004
150 mg 718/3	0,095	0,06	0,097	nd	0,05	57,5	0,007	0,025	0,004
150 mg 859/3	0,08	0,11	0,086	nd	0,06	55,8	0,0071	0,027	0,002
150 mg 860/3	0,37	0,02	0,449	nd	0,03	61,4	0,0048	0,023	0,042
150 mg 880/3	0,165	0,05	0,11	nd	0,065	58,4	0,0064	0,025	0,007
150 mg 890/3	0,07	0,05	0,118	nd	0,054	57,6	0,0062	0,025	0,002
150 mg 911/3	0,17	0,025	0,086	nd	0,056	58,9	0,007	0,021	0,002
n	*12*	*12*	*12*	*0*	*12*	*12*	*12*	*12*	*12*
mean	*0,133*	*0,081*	*0,147*		*0,052*	*58,60*	*0,0064*	*0,025*	*0,00833*
SD	*0,0898*	*0,0560*	*0,1060*		*0,0097*	*3,169*	*0,0008*	*0,0021*	*0,01100*
median	*0,0975*	*0,065*	*0,108*		*0,055*	*58*	*0,0067*	*0,025*	*0,0045*
min	*0,05*	*0,02*	*0,085*		*0,03*	*54,1*	*0,0048*	*0,021*	*0,002*
max	*0,37*	*0,23*	*0,449*		*0,065*	*65,1*	*0,0073*	*0,028*	*0,042*

	Cu	Fe	Li	Mg	Mn	Mo	Ni	P	Pb
150 mg 050/1	0,577	606	0,0066	36,6	0,047	0,007	0,064	590	0,511
150 mg 159/1	0,568	538	0,0063	36,5	0,036	0,007	0,042	510	0,502
150 mg 171/1	0,571	543	0,0062	35	0,044	0,013	0,066	576	0,768
150 mg 222/1	0,562	495	0,0081	40,2	0,031	0,014	0,12	584	0,24
150 mg 225/1	0,576	700	0,0067	43,1	0,061	0,006	0,096	576	0,23
150 mg 228/1	0,651	497	0,0068	43,2	0,046	0,008	0,029	561	0,557
150 mg 718/1	0,554	622	0,0056	33,6	0,033	nd	0,037	518	0,388
150 mg 859/1	0,634	576	0,0067	35,8	0,038	0,004	0,04	506	0,104
150 mg 860/1	0,571	565	0,0068	37	0,068	0,056	0,152	542	0,853
150 mg 880/1	0,569	537	0,012	37,5	0,028	0,003	0,05	518	0,168
150 mg 890/1	0,679	636	0,0072	39	0,055	0,003	0,047	540	0,383
150 mg 911/1	0,583	601	0,0072	36,9	0,042	0,008	0,078	512	0,786
n	*12*	*12*	*12*	*12*	*12*	*11*	*12*	*12*	*12*
mean	*0,59125*	*576,3*	*0,00718*	*37,87*	*0,04408*	*0,0117*	*0,06842*	*544,4*	*0,4575*
SD	*0,04011*	*59,99*	*0,00163*	*2,993*	*0,01224*	*0,01511*	*0,03738*	*31,69*	*0,25112*
median	*0,5735*	*570,5*	*0,00675*	*36,95*	*0,043*	*0,007*	*0,057*	*541*	*0,445*
min	*0,554*	*495*	*0,0056*	*33,6*	*0,028*	*0,003*	*0,029*	*506*	*0,104*
max	*0,679*	*700*	*0,012*	*43,2*	*0,068*	*0,056*	*0,152*	*590*	*0,853*
150 mg 050/2	0,615	628	0,0015	37,1	0,048	0,025	0,072	569	0,114
150 mg 159/2	0,635	671	0,0012	41,5	0,0377	0,007	0,037	581	0,074
150 mg 171/2	0,63	546	0,0021	41,6	0,029	0,027	0,029	489	0,192
150 mg 222/2	0,576	518	0,0016	38,7	0,024	0,025	0,047	544	0,093
150 mg 225/2	0,591	563	0,0016	39,8	0,043	0,007	0,029	500	1,52
150 mg 228/2	0,615	537	0,0015	44,8	0,031	0,013	0,115	552	0,074
150 mg 718/2	0,544	516	0,0012	34,2	0,033	0,007	0,029	481	0,094
150 mg 859/2	0,617	481	0,0013	32,6	0,026	0,009	0,148	515	0,068
150 mg 860/2	0,575	423	0,0015	36,4	0,0345	0,01	0,043	466	0,088
150 mg 880/2	0,589	643	0,0013	38,6	0,035	0,011	0,061	563	0,185
150 mg 890/2	0,541	579	0,0015	36,1	0,046	0,009	0,067	525	0,203
150 mg 911/2	0,565	822	0,0012	43,3	0,047	0,014	0,038	606	1,71
n	*12*	*12*	*12*	*12*	*12*	*12*	*12*	*12*	*12*
mean	*0,59108*	*577,3*	*0,00146*	*38,73*	*0,03618*	*0,0137*	*0,05958*	*532,6*	*0,3679*
SD	*0,03184*	*103,88*	*0,00025*	*3,667*	*0,00824*	*0,00758*	*0,03731*	*43,60*	*0,58591*
median	*0,59*	*554,5*	*0,0015*	*38,65*	*0,03475*	*0,0105*	*0,045*	*534,5*	*0,104*
min	*0,541*	*423*	*0,0012*	*32,6*	*0,024*	*0,007*	*0,029*	*466*	*0,068*
max	*0,635*	*822*	*0,0021*	*44,8*	*0,048*	*0,027*	*0,148*	*606*	*1,71*
150 mg 050/3	0,607	642	0,0016	38,6	0,061	0,006	0,058	571	0,11
150 mg 159/3	0,574	572	0,0011	37,7	0,028	0,005	0,344	510	0,257
150 mg 171/3	0,582	505	0,0018	37,2	0,025	0,016	0,065	501	0,071
150 mg 222/3	0,602	613	0,0017	41,9	0,024	0,007	0,031	524	0,184
150 mg 225/3	0,607	627	0,0013	41,9	0,045	0,011	0,034	516	0,107
150 mg 228/3	0,612	498	0,0011	41,6	0,042	0,006	0,025	481	0,348
150 mg 718/3	0,566	636	0,0013	37,5	0,027	nd	0,089	516	0,426
150 mg 859/3	0,619	682	0,001	35,9	0,034	nd	0,084	507	0,401
150 mg 860/3	0,497	472	0,0034	34,6	0,047	0,015	0,342	475	0,124
150 mg 880/3	0,609	613	0,0011	40,4	0,035	0,003	0,073	515	0,228
150 mg 890/3	0,564	609	0,0015	36,2	0,045	nd	0,037	491	0,296
150 mg 911/3	0,553	570	0,0014	37	0,033	0,004	0,036	503	0,312
n	*12*	*12*	*12*	*12*	*12*	*9*	*12*	*12*	*12*
mean	*0,58267*	*586,6*	*0,00153*	*38,38*	*0,03717*	*0,0081*	*0,1015*	*509,2*	*0,2387*
SD	*0,03484*	*64,86*	*0,00064*	*2,505*	*0,01109*	*0,00476*	*0,11480*	*24,44*	*0,12085*
median	*0,592*	*611*	*0,00135*	*37,6*	*0,0345*	*0,006*	*0,0615*	*508,5*	*0,2425*
min	*0,497*	*472*	*0,001*	*34,6*	*0,024*	*0,003*	*0,025*	*475*	*0,071*
max	*0,619*	*682*	*0,0034*	*41,9*	*0,061*	*0,016*	*0,344*	*571*	*0,426*

	Sb	Se	Si	Sn	Sr	Te	Ti	Tl	V
150 mg 050/1	0,026	0,360145	0,361111	0,2	0,035	nd	nd	0,0068	0,0138
150 mg 159/1	0,02	0,289855	0,047619	0,04	0,03	nd	nd	0,0038	0,0275
150 mg 171/1	0,02	0,284783	0,690476	0,47	0,05	nd	nd	0,013	0,0082
150 mg 222/1	0,02	0,32971	0,135714	0,04	0,046	nd	nd	0,0035	0,0225
150 mg 225/1	0,026	0,336232	0,170635	0,038	0,038	nd	nd	0,0083	0,0243
150 mg 228/1	nd	0,3	0,336508	0,28	0,05	nd	nd	0,01	0,0062
150 mg 718/1	0,03	0,344203	0,290476	0,047	0,031	nd	nd	0,0041	0,0148
150 mg 859/1	0,02	0,338406	0,714286	0,12	0,036	nd	nd	0,0083	0,0039
150 mg 860/1	0,02	0,327536	0,219841	0,23	0,039	nd	nd	0,006	0,0104
150 mg 880/1	nd	0,334058	0,131746	0,16	0,036	nd	nd	0,004	0,0024
150 mg 890/1	0,024	0,276087	0,222222	0,28	0,037	nd	nd	0,011	0,0055
150 mg 911/1	0,02	0,330435	0,140476	0,125	0,037	nd	nd	0,0075	0,0041
n	*10*	*12*	*12*	*12*	*12*	*0*	*0*	*12*	*12*
mean	*0,0226*	*0,32095*	*0,28843*	*0,16917*	*0,03875*			*0,00719*	*0,01197*
SD	*0,00366*	*0,0265*	*0,21348*	*0,13131*	*0,00659*			*0,00309*	*0,00867*
median	*0,02*	*0,33007*	*0,22103*	*0,1425*	*0,037*			*0,00715*	*0,0093*
min	*0,02*	*0,27609*	*0,04762*	*0,038*	*0,03*			*0,0035*	*0,0024*
max	*0,03*	*0,36014*	*0,71429*	*0,47*	*0,05*			*0,013*	*0,0275*
150 mg 050/2	0,02	0,434783	0,5	0,047	0,031	nd	nd	0,005	0,015
150 mg 159/2	0,026	0,481159	0,306349	0,035	0,029	nd	nd	0,003	0,0089
150 mg 171/2	0,02	0,186232	0,46746	0,04	0,041	nd	nd	0,015	0,0078
150 mg 222/2	0,02	0,4	0,302381	0,028	0,05	nd	nd	0,0064	0,0096
150 mg 225/2	0,02	0,364493	0,420635	0,03	0,042	nd	nd	0,014	0,018
150 mg 228/2	nd	0,387681	0,32619	0,037	0,052	nd	nd	0,0034	0,0062
150 mg 718/2	nd	0,354348	0,336508	0,036	0,039	nd	nd	0,0063	0,0084
150 mg 859/2	nd	0,428986	0,15873	0,04	0,037	nd	nd	0,0055	0,0088
150 mg 860/2	nd	0,321739	0,568254	0,02	0,037	nd	nd	0,008	0,0088
150 mg 880/2	0,02	0,394203	0,438889	0,06	0,031	nd	nd	0,006	0,0077
150 mg 890/2	0,02	0,404348	0,484127	0,05	0,039	nd	nd	0,0056	0,0069
150 mg 911/2	0,03	0,513043	0,347619	0,047	0,025	nd	nd	0,0042	0,011
n	*8*	*12*	*12*	*12*	*12*	*0*	*0*	*12*	*12*
mean	*0,022*	*0,38925*	*0,3881*	*0,03917*	*0,03775*			*0,00687*	*0,00976*
SD	*0,00385*	*0,08279*	*0,11237*	*0,01080*	*0,00807*			*0,00382*	*0,00344*
median	*0,02*	*0,3971*	*0,38413*	*0,0385*	*0,038*			*0,0058*	*0,0088*
min	*0,02*	*0,18623*	*0,15873*	*0,02*	*0,025*			*0,003*	*0,0062*
max	*0,03*	*0,51304*	*0,56825*	*0,06*	*0,052*			*0,015*	*0,018*
150 mg 050/3	0,02	0,542029	0,404762	0,048	0,029	nd	nd	0,009	0,016
150 mg 159/3	0,02	0,468116	0,161111	0,04	0,03	nd	nd	0,0036	0,026
150 mg 171/3		0,44058	0,279365	0,03	0,043	nd	nd	0,003	0,0096
150 mg 222/3	0,02	0,468841	0,642857	0,04	0,044	nd	nd	0,0076	0,0049
150 mg 225/3	0,025	0,048768	0,245238	0,026	0,035	nd	nd	0,0038	0,0403
150 mg 228/3	nd	0,392029	0,35	0,023	0,047	nd	nd	0,0028	0,0074
150 mg 718/3	0,027	0,443478	0,346825	0,048	0,035	nd	nd	0,0052	0,0118
150 mg 859/3	0,03	0,358696	0,238095	0,025	0,033	nd	nd	0,0051	0,005
150 mg 860/3	nd	0,308696	0,555556	0,044	0,061	nd	nd	0,02	0,0049
150 mg 880/3	0,026	0,386957	0,587302	0,05	0,033	nd	nd	0,006	0,0056
150 mg 890/3	0,02	0,343478	0,357937	0,14	0,032	nd	nd	0,0043	0,0046
150 mg 911/3	0,02	0,396377	0,277778	0,03	0,033	nd	nd	0,01	0,0075
n	*9*	*12*	*12*	*12*	*12*	*0*	*0*	*12*	*12*
mean	*0,02311*	*0,38317*	*0,37057*	*0,04533*	*0,03792*			*0,00670*	*0,01197*
SD	*0,00392*	*0,12304*	*0,15127*	*0,03135*	*0,00927*			*0,00479*	*0,01089*
median	*0,02*	*0,3942*	*0,34841*	*0,04*	*0,034*			*0,00515*	*0,00745*
min	*0,02*	*0,04877*	*0,16111*	*0,023*	*0,029*			*0,0028*	*0,0046*
max	*0,03*	*0,54203*	*0,64286*	*0,14*	*0,061*			*0,02*	*0,0403*

	Zn
150 mg 050/1	3,7692308
150 mg 159/1	3,7948718
150 mg 171/1	3,3760684
150 mg 222/1	3,4017094
150 mg 225/1	3,9230769
150 mg 228/1	3,5982906
150 mg 718/1	3,5128205
150 mg 859/1	3,1794872
150 mg 860/1	2,974359
150 mg 880/1	3,1025641
150 mg 890/1	4,008547
150 mg 911/1	3,7350427
n	*12*
mean	*3,5313*
SD	*0,3320*
median	*3,5555556*
min	*2,974359*
max	*4,008547*
150 mg 050/2	3,6923077
150 mg 159/2	3,6837607
150 mg 171/2	3,5299145
150 mg 222/2	2,9487179
150 mg 225/2	2,9145299
150 mg 228/2	3,6068376
150 mg 718/2	3,0854701
150 mg 859/2	3,042735
150 mg 860/2	2,7179487
150 mg 880/2	3,4529915
150 mg 890/2	3,6837607
150 mg 911/2	4,6837607
n	*12*
mean	*3,4202*
SD	*0,5294*
median	*3,491453*
min	*2,7179487*
max	*4,6837607*
150 mg 050/3	4,2051282
150 mg 159/3	3,6153846
150 mg 171/3	3,2478632
150 mg 222/3	3,3846154
150 mg 225/3	3,6666667
150 mg 228/3	3,2564103
150 mg 718/3	3,5641026
150 mg 859/3	3,2307692
150 mg 860/3	2,7264957
150 mg 880/3	3,4358974
150 mg 890/3	3,5384615
150 mg 911/3	3,7435897
n	*12*
mean	*3,4679*
SD	*0,3570*
median	*3,4871795*
min	*2,7264957*
max	*4,2051282*

	Al	As	Ba	Be	Bi	Ca	Cd	Co	Cr
control/1-150/1	0,17	0,19	0,08		0,44	0,96	0,36	0,40	0,30
control/2-150/2	0,88	0,21	0,96		0,63	0,73	0,99	0,32	0,34
control/3-150/3	0,33	0,80	0,16		0,43	0,07	0,45	0,83	0,55
control1-2	0,94	0,07	0,04		0,81	0,13	0,45	0,36	0,21
control1-3	0,15	0,01	0,02		0,36	0,75	0,18	0,40	0,97
control 2-3	0,33	0,52	0,59		0,23	0,49	0,35	0,16	0,23
control1		*0,117*	*0,065*						
control2		*0,090*	*0,112*						
control3		*0,076*	*0,100*						
		means	means						
		0,094	0,092	common (united) control					

	Cu	Fe	Li	Mg	Mn	Mo	Ni	P	Pb
control/1-150/1	0,48	0,41	0,82	0,93	0,11	0,26	0,10	0,46	0,38
control/2-150/2	0,45	0,97	0,39	0,87	0,38	0,85	0,47	0,48	0,41
control/3-150/3	0,26	0,62	0,39	0,95	0,24	0,86	0,09	0,34	0,29
control1-2	0,79	0,29	0,00004	0,60	0,33	0,48	0,83	0,59	0,19
control1-3	0,57	0,09	0,00002	0,63	0,10	0,73	0,82	0,43	0,04
control 2-3	0,68	0,28	0,17	0,91	0,97	0,28	0,34	0,96	0,52
control1			*0,0074*						*0,3627*
control2			*0,00171*						*0,2193*
control3			*0,00135*						*0,1634*
			means						means
			0,00349						0,24843

	Sb	Se	Si	Sn	Sr	Te	Tl	Ti	V
control/1-150/1	0,34	0,02	0,89	0,45	0,53			0,36	0,007
control/2-150/2	0,66	0,003	0,27	0,43	0,16			0,62	0,00000
control/3-150/3	0,64	0,09	0,93	0,44	0,61			0,48	0,004
control1-2	0,33	0,29	0,90	0,008	0,31			0,33	0,93
control1-3	0,35	0,02	0,67	0,003	0,95			0,49	0,63
control 2-3	0,41	0,046	0,57	0,19	0,40			0,14	0,70
control1		*0,28949*		*0,13367*			n=6		*0,00087*
control2		*0,30326*		*0,04308*			n=7		*0,00077*
control3		*0,3186*		*0,03758*			n=10		*0,00078*
									átlagok
		0,30378		0,07144			n=12	150/1	*0,01197*
								150/2	*0,00976*
	150/1	*0,32095*						150/3	*0,01197*
	150/2	*0,38925*							
	150/3	*0,38317*							
		means							
		0,12	150/1				150/1		4,48E-07
		5,78E-06	150/2				150/2		*3,9E-14*
		0,000884	150/3				150/3		*1,9E-05*

compared to united control means

compared to united control means

	Zn
control/1-150/1	0,56
control/2-150/2	0,83
control/3-150/3	0,91
control1-2	0,87
control1-3	0,59
control 2-3	0,08
control1	
control2	
control3	